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ON THE NON-INFLUENCE OF RISE IN BODY TEMPERATURE INDUCED BY DRUGS UPON THE PROTEIN QUOTIENT AND THE ENUMERATION OF WHITE CORPUSCLES

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The artificial raising of body temperature in the following experiments was undertaken for the purpose of finding out what effect such a rise in temperature might have on the globulins and total proteins of the blood and what variations, if any, might be produced in the enumeration and type of the white corpuscles.

The drugs used in these experiments were the fluid extract of ergot and a saturated solution of calcium lactate.

The use of ergot and the derivatives of ergot for the purpose of raising temperatures has been very limited, the only article dwelling particularly on this characteristic of the drug being that of T. S. Githens, although it has been referred to by two or three others. Githens (1) used the ergotoxin phosphate of the Burroughs Wellcome Company in all his experiments; but, having employed the fluid extract empirically to bring on artificial paroxysms in malaria without deleterious effect to patients, we deemed it a less toxic preparation and therefore more suitable for our special purpose. We used Squibb and Sons' fluid extract of ergot.

Rabbits were the animals employed. The drug was administered intravenously in the lateral or central ear vein. The rabbits were left free in their cages and the marked restlessness noted by Githens (1) was not observed in our animals, not even in those receiving toxic doses. This may have been due to the different

preparation or to the alcohol present in the fluid extract of which very little remained after the sterilization.

The rabbits remained extremely quiet, being less restless than normal, sitting hunched up in a corner of the cage and having the appearance of sick animals. Slight muscular tremors and chewing movements were, aside from the extreme rapidity in breathing, all the external manifestations present in the animals receiving less than toxic doses. The rabbits receiving fatal doses died either immediately on receiving the intravenous injection, or in clonic convulsions following directly on the injecting and ending in death within twenty minutes. The difference in the rise in temperature between a toxic or a lethal dose (2 cc. per kilogram weight) and a non-toxic dose (1.5 cc. per kilogram weight) was very slight—not over 0.2° .

Fluid extract of ergot, 1.5 cc. per kilogram weight, seemed to produce more effect on some of the rabbits than on others. Rabbit III, on receiving this amount gave several convulsive kicks, followed by utter prostration with cold extremities, which remained cold until his temperature had risen considerably. Respiration and heart-beat were perceptibly slower than normal and his rise in temperature rather delayed. After his temperature had risen recovery was quite rapid, the rapidity of his breathing increasing until over 300 respirations per minute. The other rabbits receiving this amount (1.5 cc. per kilogram weight) appeared to suffer very little inconvenience other than a temporary languor, with the appearance of a sick rabbit and the rapidity of breathing. They were rather disinclined to move and the ears of some were cold.

As no precedent in the dosage of fluid extract of ergot for raising temperatures has been established, we gave to rabbits of differing weights 2 cc. each, and noted the results. In all of the tables given the temperatures are Centigrade and taken per rectum

February 26, 1918

Rabbit.....	I, wt. 2.5 kg. 39.80°	II, wt. 2.8 kg. 39.50°	III, wt. 3.2 kg. 39.50°
Normal temperature.....			
10.45 a.m. Injected 2 cc. fluid extract ergot into each rabbit			
12.00 a.m.....	41.65°	41.75°	40.30°
1.00 p.m.....	40.50°	41.00°	40.70°
2.00 p.m.....	40.40°	40.35°	40.50°
3.00 p.m.....	40.10°	39.60°	40.10°

1.00 p.m. I and II, slight muscular and chewing movements; III, growling.
 3.00 p.m. chewing movements still going on, tremors have ceased.

March 4, 1918

Rabbit.....	I, wt. 2.5 kg. 39.50°	II, wt. 2.8 kg. 39.50°	III, wt. 3.2 kg. 39.00°
Normal temperature.....			
11.00 a.m. injected 1 cc. ergot per kilogram weight			
12.22 p.m.....	41.10°	40.90°	39.50°
1.10 p.m.....	40.10°	41.00°	40.90°
2.10 p.m.....	40.00°	40.30°	40.30°

March 11, 1918

Rabbit.....	I, wt. 2.5 kg. 39.40°	II, wt. 2.8 kg. 39.50°	III, wt. 3.2 kg. 39.50°	IV, wt. 2.8 kg. —
Normal temperature.....				
10.30 a.m. Injected 2 cc. ergot per kilogram weight				11.00 Injected 2 cc. per kilogram weight.
	Died immediately			Died in 20 minutes. Clonic convulsions.
12.22 p.m.....	—	41.70° Prostration	40.90° Prostration	—
1.10 p.m.....	—	40.90°	41.20°	—
2.10 p.m.....	—	40.10°	40.30°	—

This dose was considered too large, so we increased our dosage more gradually.

March 18, 1918

Rabbit	II, wt. 2.8 kg. 39.10°	III, wt. 3.2 kg. 39.40°	V, wt. 4.02 kg. 39.30°
Initial temperature			
10.30 a.m. Injected 1 cc. ergot per kilogram weight			
11.25 a.m.....	39.00°	39.40°	39.70°
11.50 a.m.....	39.70°	39.70°	40.35°
12.40 p.m.....	40.40°	40.30°	40.50°
1.55 p.m.....	39.80°	39.60°	40.05°

March 22, 1918

Rabbit.....	III, wt. 3.2 kg. 39.20°	V, wt. 4.02 kg. 39.30°
Initial temperature.....		
10.20 a.m. Injected 1.25 cc. fluid extract ergot per kilogram weight		
11.00 a.m.....	39.40°	39.50°
11.30 a.m.....	39.60°	40.40°
12.00 m.....	40.15°	40.85°
12.27 p.m.....	40.50°	41.10°
1.00 p.m.....	40.90°	41.00°
1.35 p.m.....	40.70°	40.90°
2.00 p.m.....	40.40°	40.40°

Nothing abnormal was observed in the rabbits receiving this dose, except slight acceleration in breathing and rise in temperature noted. Rabbits were feeding.

April 1, 1918

Rabbit.....	III, wt. 3.2 kg. 39.55°	V, wt. 4.02 kg. 39.40°	VI, wt. 3.6 kg. 39.50°
Initial temperature.....			
10.40 a.m. Injected 1.5 cc. fluid extract ergot per kilogram weight	Gave several con- vulsive kicks followed by utter prostra- tion, with cold extremities; respiration and heart-beat perceptibly slower.	"Sick" These rabbits exhibited no marked symp- toms; ap- peared languid and disinclined to move. Ears seemed cold.	"Sick"

April 1, 1918—Continued

Rabbit	III, wt. 3.2 kg. 39.55°	V, wt. 4.02 kg. 39.40°	VI, wt. 3.6 kg. 39.50°
Initial temperature			
11.25 a.m.	39.40° Partially recovered.		
11.45 a.m.	39.90°	40.20°	40.30°
12.20 p.m.	40.55°	41.05°	41.05°
1.00 p.m.	41.10°	41.50°	41.65°

Respiration of all these rabbits very rapid, simulating tremors.

	Respiration 320	Respiration 260	Respiration 230
1.30 p.m.	41.40°	41.60°	41.70°
2.00 p.m.	40.70°	41.50°	41.60°
2.45 p.m.	40.60°	41.40°	41.60°
3.20 p.m.	40.50°	41.20°	41.00°

3.30 p.m. Temperatures going down. Breathing still very rapid: Shaking entire body; rabbits sitting hunched together.

4.30 p.m. Rabbits feeding; respiration almost normal. 1.5 cc. per kilogram wt. was considered the maximum non-toxic dose and was the amount used in the rest of the experimentation.

The rabbit taken for the globulin work was a male (VII) weighing 3.6 kg. He was injected each time with 1.5 cc. fluid extract ergot per kilogram weight. He appeared to suffer very little inconvenience from this dosage other than a temporary languor.

His rise in temperature was accompanied by marked rapidity in breathing; temperature rise 41.60° to 41.75°; respiration reached as high as 300 per minute.

He acquired no tolerance for the drug, appearing rather limp at the end of the injection, so no attempt was made to further increase the dose.

This rabbit had been employed previously for globulin and total protein work and his normals were well established. The conditions and feeding were kept as uniform as possible, as they were with all rabbits made use of in these experiments.

His normal range was as follows:

NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT OF GLOBULIN IN TOTAL PROTEIN
1.27	4.09	2.00	6.09	32.0
1.50	4.65	1.37	6.02	22.0

He was injected each time with 1.5 cc. per kilogram weight and as he lost practically no weight during the period of experimentation, the amount given was practically the same each time; he was weighed before each injection. No deleterious effects were observed from frequent administration of the drug.

The blood for the globulin work was drawn two hours after injecting when the rise in temperature was highest. In those experiments in which the globulins were determined on the following day the time was twenty-four hours later.

The method followed was the refractometric method of Prof. T. B. Robertson (2).

Rabbit VII

DATE	NON- PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT OF GLOBULIN IN TOTAL PROTEIN	RATIO OF GLOBULIN TO ALBUMIN
April 1.....	1.3	4.3	1.6	5.9	27	1:2.4
April 5.....	1.5	4.1	1.7	5.8	29	1:2.7
April 8.....	1.4	4.4	1.3	5.7	22	1:3.3
April 12.....	1.5	4.2	1.5	5.7	26	1:2.8
April 15.....	1.4	4.5	1.6	6.1	26	1:2.8
April 16.....	1.3	4.2	1.6	5.8	27	1:2.6
April 18.....	1.3	4.3	1.7	6.0	27	1:2.5
April 19.....	1.5	4.1	1.5	5.6	26	1:2.7

No deviations from the normal were observed in this series of experiments, so we may draw the conclusion that the artificial rise in temperature caused by injections of fluid extract of ergot has no marked effect on the globulin or total proteins of the blood.

The white count was made on the same days the globulin work was done.

The normal range of rabbit VII was as follows:

TIME		
10.30 a.m.....	11,200	10,800
11.00 a.m.....	10,000	9,800
12.00 m.....	9,200	9,600
1.00 p.m.....	10,200	10,600
2.00 p.m.....	9,800	11,100
3.00 p.m.....	9,700	9,600
4.00 p.m.....	9,900	9,600

The range noted after injection did not differ materially from the normal range. The counts were made on each day of the globulin determination and differed so little that a few characteristic counts only are submitted.

April 12, 1918

10.00 a.m. Before injection.....	10,400
10.30 a.m. Injected.	
11.00 a.m.....	11,200
12.00 m.....	10,400
2.00 p.m.....	9,800
3.00 p.m.....	10,000

April 15, 1918

10.00 a.m.....	11,000
10.30 a.m. Injected	
11.00 a.m.....	10,200
11.30 a.m.....	10,600
12.00 m.....	10,000
1.30 p.m.....	9,800
2.00 p.m.....	10,400
2.30 p.m.....	10,200
3.00 p.m.....	9,600
4.00 p.m.....	10,600

April 16, 1918

10.00 a.m.....	12,000
11.00 a.m.....	10,200
12.00 m.....	11,400
1.30 p.m.....	9,800
2.00 p.m.....	10,600
2.30 p.m.....	10,200
3.00 p.m.....	9,800

We concluded from these counts that the rise in temperature induced by fluid extract of ergot was not accompanied by a corresponding increase in the numbers of white corpuscles.

It has been shown by Bosc and Vedel (3), Fornaro and Micheli (4), Schaps (5), Gofferye (6), Meyer and Reitschel (7), T. C. Burnett (8) and others that the subcutaneous injection of sodium chloride solutions causes a rise of temperature in animals and in man. As the rise caused by sodium chloride is insufficient for our purpose in endeavoring to find a salt which would be efficient, we made some rather interesting experiments with calcium lac-

tate, upon the pharmacology of which very little work has been reported.

The calcium lactate experiments were carried out simultaneously with the ergot. A saturated solution (1: 20) of calcium lactate (c.p. Merck) in distilled, chlorine-free water was used.

The calcium lactate gave no untoward symptoms of any kind, until a dose large enough to produce the symptoms of calcium poisoning was reached. The rabbits had previously (under dosage up to 8 cc.) exhibited no other symptoms than a slight depression lasting about an hour—the duration of the fall in temperature. With the rise in temperature they regained all their usual characteristics and moved about their cages feeding as usual. During the phase of the rise in temperature nothing abnormal was observed about them.

This appeared to be characteristic of the larger doses: first a drop in temperature with the accompanying lassitude, followed by the rise in temperature and the apparent return to normal.

On injecting 10 cc. of saturated (5 per cent) solution per kilogram weight, one of the rabbits died and the one that recovered exhibited all the symptoms of calcium poisoning. Rabbit X had been receiving injections of 8 cc. of calcium lactate without exhibiting any untoward symptoms. He received 10 cc. per kilogram weight (weight 4.1 kg.) at 12.00 m. On lifting him out of the holding-box he was seen to be in a greatly depressed condition—pupils contracted and all the symptoms of extreme exhaustion. Under artificial stimulation he rallied and at 12.20 was able to stand and appeared in better condition. At 12.40 he was lying down, breathing and heart-beat perceptibly slower; he could not be aroused again—artificial stimulation had no effect and at 1.15 p.m. he died. The blood for globulin work was taken at 12.50 p.m. when it was seen that the animal would not recover.

The other rabbit, IX, receiving the same amount (10 cc. per kilogram weight) at 11.30 a.m. was not so depressed when taken from the holding-box; at 12.10 he was lying on his side, breathing and heart-beat very slow and exhaustion profound. About half an hour later he was sitting with hind legs extended, his depression still very great. An hour and a half after injecting,

his temperature was still sub-normal, and although sitting in a normal position, he was very quiet and limp.

He refused all food for twenty-four hours and although apparently recovered, he was very ugly when approached, growling and attempting to bite. On the third day after this injection he had entirely recovered, accepted food and made no further attempts to resist handling. We were rather surprised at this extreme difference, as 9 cc. calcium lactate per kilogram weight had practically no greater effect than 8 cc. per kilogram weight.

Here as with the fluid extract of ergot, having no established dose to go by, we injected each rabbit used, regardless of weight, with 5 cc. of the (1:20) calcium lactate solution.

The sterile solution was injected in the middle or marginal vein of the ear, temperature taken per rectum as before.

February 26, 1918

Rabbit	V, wt. 4.02 kg.	VIII, wt. 3.2 kg.	IX, wt. 2.8 kg.
Initial temperature	39.10°	40.00°	39.20°
11.00 a.m. Injected each rabbit with 5 cc. calcium lactate			
12.00 m.....	40.30°	41.15°	39.70°
1.00 p.m.....	40.20°	40.50°	40.00°
2.00 p.m.....	40.30°	41.00°	40.70°
3.00 p.m.....	39.75°	40.70°	40.20°

As no untoward effects occurred and a very slight rise in temperature was observed, we injected our next set with 3 cc. per kilogram weight.

March 4, 1918

Rabbit	V, wt. 4.02 kg.	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature	39.50°	39.30°	39.10°
11.30 a.m. Injected 3 cc. per kilo- gram weight of each rabbit			
12.20 p.m.....	41.10°	40.40°	39.00°
1.10 p.m.....	40.10°	40.00°	39.00°
2.10 p.m.....	40.00°	39.60°	39.20°

No untoward effects were noted, the drug apparently having no effect whatsoever on the animals except that the slight rise in temperature was further increased.

March 11, 1918

Rabbit.....	IV, wt. 4.02 kg.	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature	39.00°	39.30°	39.10°
11.30 a.m. Injected 4 cc. calcium lactate per kilogram weight of each rabbit			
1.15 p.m.....	40.40°	40.00°	39.00°
2.15 p.m.....	39.60°	40.10°	39.20°

Transferred rabbit IV to ergot group.

March 18, 1918

Rabbit.....	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature	39.00°	39.10°
10.20 a.m. Injected 5 cc. calcium lactate per kilogram weight of each rabbit		
11.00 a.m.....	39.00°	39.00°
11.20 a.m.....	38.80°	38.60°
12.45 p.m.....	39.90°	39.10°
2.05 p.m.....	40.30°	38.60°

Although with this amount (5 cc. per kilogram weight) we had not as yet succeeded in obtaining the desired rise in temperature, the drop in temperature during the first hour after injection was noted. This held in the following experimentation.

March 25, 1918

Rabbit.....	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature.....	39.10°	39.00°
10.45 a.m. Injected 6 cc. calcium lactate per kilogram weight of each rabbit		
11.15 a.m.....	38.40°	38.50°
11.45 a.m.....	39.40°	39.30°
12.10 p.m.....	39.50°	39.40°
12.35 p.m.....	39.95°	39.50°
1.05 p.m.....	38.80°	40.30°
1.40 p.m.....	39.00°	40.70°
2.15 p.m.....	39.00°	40.75°
2.40 p.m.....	—	40.85°
3.10 p.m.....	—	40.65°
4.00 p.m.....	—	39.80°

April 1, 1918

Rabbit.....	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature.....	39.20°	39.10°
10.30 a.m. Injected 7 cc. calcium lactate per kilogram weight of each rabbit		
11.00 a.m.....	38.60°	38.60°
11.30 a.m.....	38.90°	39.00°
12.15 p.m.....	39.55°	39.65°
1.05 p.m.....	40.50°	40.55°
1.40 p.m.....	40.80°	40.75°
2.15 p.m.....	41.10°	40.70°
2.50 p.m.....	41.40°	41.00°
3.30 p.m.....	41.00°	40.70°

At this amount (7 cc.) there were absolutely no visible symptoms accompanying the rise in temperature; the animals running free in their cages, were feeding and to all appearances perfectly normal.

April 5, 1918

Rabbit.....	VIII, wt. 3.2 kg.	IX, wt. 2.83 kg.
Initial temperature.....	39.00°	39.10°
10.30 a.m. Injected 8 cc. calcium lactate per kilogram weight of each rabbit		
11.00 a.m.....	38.50°	38.70°
11.30 a.m.....	38.60°	38.90°
12.00 m.....	39.00°	39.20°
12.30 p.m.....	39.60°	39.50°
1.10 p.m.....	40.40°	40.50°
2.00 p.m.....	41.20°	41.00°
2.30 p.m.....	41.50°	41.40°
3.00 p.m.....	41.20°	41.20°
3.30 p.m.....	40.95°	40.90°
4.00 p.m.....	40.40°	40.10°

The rabbits at this amount exhibited a slight lassitude for about an hour; when disturbed they moved freely about but were disinclined to move of their own volition. This lassitude seemed to correspond directly with the drop in temperature. Of the (1:20) solution of calcium lactate 8 cc. was the amount injected in the work on enumeration of the white corpuscles and also for the work on the globulin and total protein content. In the last two experiments 9 cc. and 10 cc. per kilogram weight were injected; here the symptoms of calcium poisoning were noted.

April 22, 1918

Rabbit.....	X, wt. 4.1 kg. 39.30°	IX, wt. 2.83 kg. 39.10°
Initial temperature.....		
11.30 a.m. Injected 10 cc. calcium lactate per kilo- gram weight.		
12.10 p.m.....	Rabbit taken from holding- box in collapsed condi- tion. Under stimulation rallied and was able to stand	38.20° Lying on side; Breathing slow. Exhaust- ion profound
12.40 p.m.....	Lying on side in extreme exhaustion. Blood taken just before death for globulin test.	38.20° Rallied somewhat, sitting in normal position. Very quiet
1.15 p.m.....	Died	38.40°
2.15 p.m.....		39.10°
3.05 p.m.....		39.00° No rise in tempera- ture
4.05 p.m.....		

Succeeding day, rabbit IX was moving about freely in cage but growled and otherwise resisted handling. Refused all food.

April 24, 1918. Feeding; temperature 39.20°.

April 26, 1918. At the suggestion of Dr. Robertson the globulins were determined on this date (see table) and were found to have returned to normal. Temperature range between 39.10° and 39.50°; he seemed to have completely recovered and was normal in all outward respects.

Rabbits IX and X had been subjected to many globulin determinations and their normal range was well established. Condition and feeding were maintained as uniformly as possible.

Rabbit IX, normal range

NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN
1.2	4.0	2.4	6.4	38
1.5	5.2	1.3	5.5	24

Rabbit X, normal range

NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN
1.3	4.1	2.2	6.3	35
1.5	4.5	1.3	5.8	22

Rabbit X was injected with 8 cc. calcium lactate per kilogram weight and the globulins determined (by Robertson's refractometric method) in a series of experiments, several days elapsing between each determination.

His temperature reached its height four to four and a half hours after injection. His maximum temperature was 41.50°. The globulin determinations were made when his "aseptic fever" was at its height about four hours after injecting.

Rabbit X, weight 4.1 kg.; injected with 8 cc. per kilogram weight

DATE	NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN	RATIO OF GLOBULIN TO ALBUMIN
April 1.....	1.3	4.2	1.4	5.6	25	1:3.0
April 5.....	1.4	4.4	1.4	5.8	24	1:3.2
April 8.....	1.3	4.3	1.3	5.6	23	1:3.4
April 12.....	1.5	4.2	1.5	5.7	26	1:2.7

As the globulin content appeared not to vary at all from the normal, the determination was made on the day of injection and on the succeeding day as well. The dose was also increased 1 cc. per kilogram weight and rabbit IX was injected at the same time to serve as a check.

Rabbit IX, weight 2.83 kg.; 8 cc. per kilogram weight

DATE	NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN	RATIO OF GLOBULIN TO ALBUMIN
April 15.....	1.2	4.3	1.6	5.9	27	1:2.7
April 16.....	1.3	4.1	1.9	6.0	30	1:2.2

Rabbit X, weight 4.1 kg.; 8 cc. per kilogram weight

April 15.....	1.2	4.3	1.3	5.6	23	1:3.4
April 16.....	1.3	4.5	1.4	5.9	23	1:3.2

The dose of calcium lactate was increased to 9 cc. per kilogram weight. This dose gave no higher rise in temperature and no apparent greater systemic effect than 8 cc. per kilogram weight.

Rabbit IX, weight 2.83 kg.; 9 cc. per kilogram weight

DATE	NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN	RATIO OF GLOBULIN TO ALBUMIN
April 18.....	1.2	4.9	1.2	6.1	19	1:4.0
April 19.....	1.3	3.4	1.8	5.2	34	1:1.8

Rabbit X, weight 4.1 kg.; 9 cc. per kilogram weight

April 18.....	1.4	4.6	1.1	5.7	19	1:4.1
April 19.....	1.3	4.7	1.2	5.9	20	1:3.9

The amount of calcium lactate injected was next increased to 10 cc. per kilogram weight and grave systemic disturbances occurred resulting in the death of one rabbit and profound derangement of the other.

There also seemed to be an alteration in the globulins but whether or not this was due to the calcium ion must be left for later experimentation.

Rabbit IX, weight 2.83 kg.; injected 10 cc. per kilogram weight

DATE	NON-PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	PER CENT GLOBULIN IN TOTAL PROTEIN	RATIO OF GLOBULIN TO ALBUMIN
April 22, 3.30 p.m.....	1.3	4.8	0.8	5.6	14	1:6.0
April 23, 3.00 p.m.....	1.3	2.3	3.4	5.7	69*	1:0.7
April 26, 2.00 p.m.....	1.3	4.3	1.6	5.9	27	1:2.6

Rabbit X, weight 4.1 kg.; injected 10 cc. per kilogram weight at 12.00 m.

April 22, 12.50 p.m.....	1.3	5.2	0.7	5.9	11	1:7
1.15 p.m.....	Died					

* This was the only marked deviation from the normal, and we were unable to account for it.

As the temperature did not rise during the day of injection above normal, the alteration in the globulin content might be due to the disturbance of the balance of the calcium ion concentration in the body and some work along this line will be undertaken at a later date.

Excess of calcium is depressant to most nervous and muscular functions finally bringing even the heart muscle to a standstill. This effect is only obtained by intravenous injections of large amounts of calcium salts. These symptoms of depression were those exhibited by our rabbits on receiving the intravenous injection of 10 cc. calcium lactate.

The rise in temperature due to the injection of this salt had no apparent effect on the globulin content of the blood.

The white counts which were made on rabbit X showed a slight diminution in numbers immediately following the intravenous injections; as an amount of fluid large in relation to the total aggregate of blood in the rabbits was injected, this was probably due to an actual temporary dilution.

Normal count of rabbit X was as follows:

9.30 a.m.....	10,500
10.00 a.m.....	10,200
10.30 a.m.....	11,200
11.00 a.m.....	11,000
11.30 a.m.....	11,200
2.00 p.m.....	10,000
3.00 p.m.....	9,800
3.30 p.m.....	9,500
4.00 p.m.....	9,700
4.30 p.m.....	9,200
5.00 p.m.....	9,000

Rabbit X, weight 4.1 kg.; April 15, 1918

10 30 a.m.....	11,200
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Injected 8 cc. calcium lactate per kilogram weight

11.00 a.m.....	9,200
12.00 m.....	9,400
1.00 p.m.....	9,500
1.30 p.m.....	11,400
2.00 p.m.....	10,000
2.30 p.m.....	11,600
3.00 p.m.....	10,200
3.30 p.m.....	10,100
4.00 p.m.....	9,800

Rabbit X, weight 4.1 kg.; April 8, 1918

10 30 a.m.....	10,200
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Injected 8 cc. calcium lactate per kilogram weight

11.30 a.m.....	9,000
12.00 m.....	9,200
1.30 p.m.....	9,800
2.00 p.m.....	10,400
2.30 p.m.....	9,800
3.00 p.m.....	10,200
3.30 p.m.....	10,400
4.00 p.m.....	9,600
4.30 p.m.....	9,900

These counts were well within the normal range of rabbits, the only peculiarity being that already noted, of the drop immediately following the intravenous injection. The white counts usually ranged higher in the morning owing to our method of feeding the animals once a day only, early in the morning. The only exceptions to this rule were made when desirous of learning whether animals had returned to their normal status, when a cabbage leaf was given them.

SUMMARY

1. Fluid extract of ergot in doses of from 1 to 1.5 cc. per kilogram body weight administered intravenously to rabbits induced a steady rise of body temperature from 1.5° to 2.2°C. Higher doses proved fatal.

2. Calcium lactate in doses of from 5 to 8 cc. of a 1:20 solution administered intravenously to rabbits caused an initial fall of from 0.4° to 0.6°C. in temperature accompanied in the higher doses by symptoms of calcium poisoning. This was succeeded by a strong rise of from 1.5° to 2.5° and disappearance of the symptoms of poisoning. Higher doses were fatal.

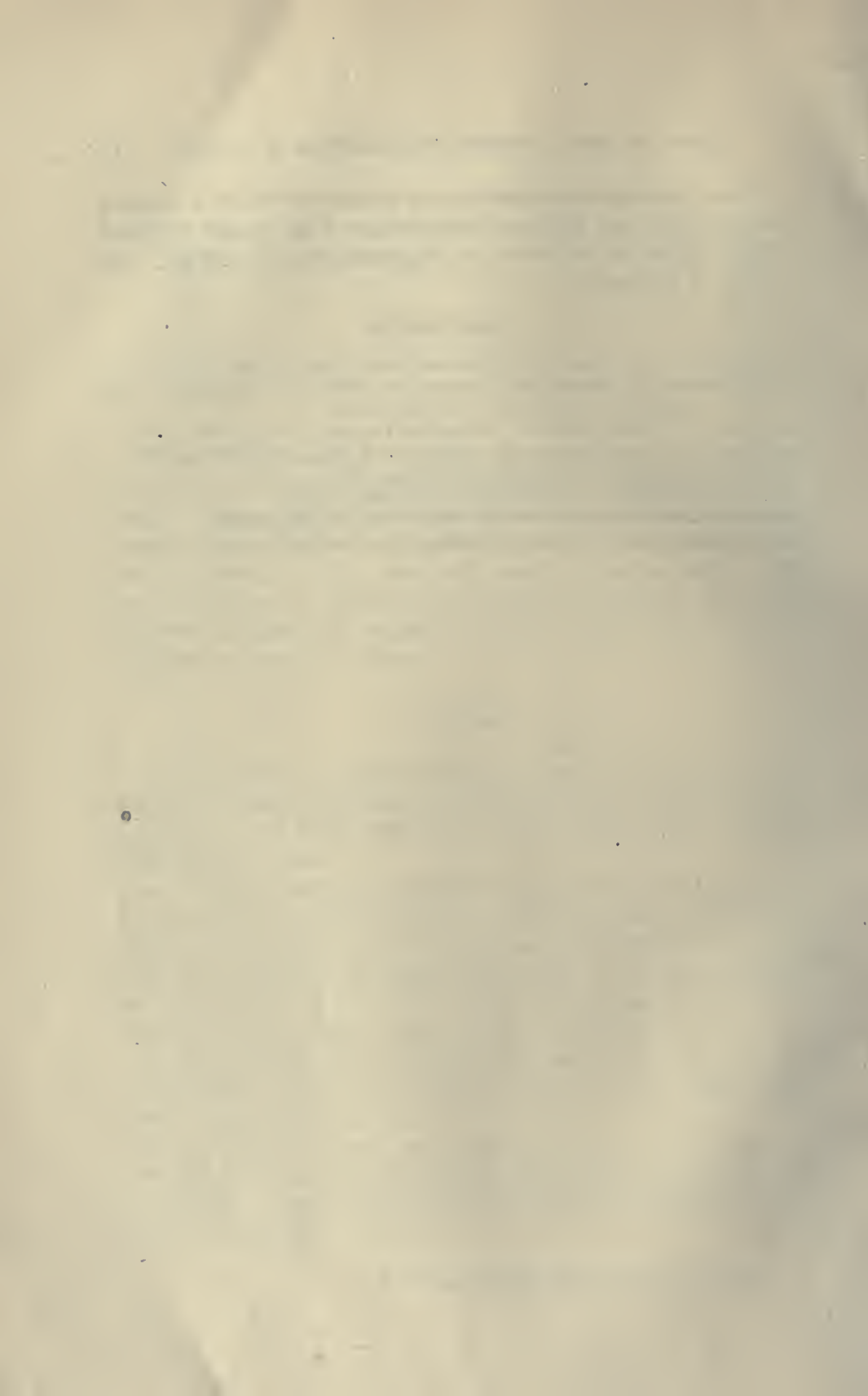
3. In sub-lethal doses, neither fluid extract of ergot nor calcium lactate caused any decisive alteration in the protein quotient or the leucocyte count.

In conclusion we may say, these experiments show that the "aseptic fever" induced by these drugs causes no alteration in the globulin content of the blood nor does any alteration of note occur in the leucocyte count.

For advice and assistance in that portion of the work relating to the raising and taking of temperatures I am greatly indebted to Dr. Lillian M. Moore of the department of physiology, University of California.

REFERENCES

- (1) GITHENS, T. S.: *Journ. of Pharm. and Exper. Ther.*, x, 1917, p. 327.
- (2) ROBERTSON, T. BRAILSFORD: *The physical chemistry of the proteins*, 1918, Longmans, Green & Co., New York, p. 61.
- (3) BOSC, F. J., AND VEDEL, V.: *Archives de Physiol.*, 5 Ser., 8, 1896, p.-937.
- (4) FORNARO, L., AND MICHELI, F.: *Arch. Ital. de. Biol.*, xxxii, 1899, p. 87.
- (5) SCHAPS: *Stuttgarter Kongress der Medizin*, 1906.
- (6) GOFFLERYE: *Jahr. f. Kinderheilk*, lxviii, 1908, p. 1309.
- (7) MEYER AND RIETSCHEL: *Berliner Klin. Woch.*, lx, 1908, p. 2217.
- (8) BURNETT, THEO. C.: *Univ. of California Pub. in Physiol.*, iv, 1910, no. 2.



THE DETOXIFYING ACTION OF SODIUM SALT ON POTASSIUM SALT IN THE GUINEA PIG

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The literature on the toxicity of urine contains the statement that a certain percentage of this toxicity is due to potassium chloride. What is meant may perhaps be understood best by an illustration. Suppose the intravenous injection of 100 cc. of urine at a given rate is fatal to a rabbit of a given weight. This urine contains, say, 1 per cent KCl. But it would require 125 cc. of a 1 per cent KCl solution to kill a rabbit of equal weight. The urine, therefore, contains 80 per cent of the fatal dose of KCl and it is concluded that 80 per cent of its toxicity is due to KCl. Such reasoning does seem primarily rather questionable, for potassium salts are not the only salts present in such a complicated mixture as urine, where various constituents might even depress a possible toxic action of potassium. Having in mind the experiments of J. Loeb and others on balanced salt solutions, we undertook a series of experiments with the object to test whether the presence of another salt in a solution of potassium salt exercised any influence on the toxicity of the latter, on intravenous injection in a warm-blooded animal. The experiments were done on guinea pigs and we used preferably animals weighing approximately 200 grams. The fatal dose of KCl is given by Aaronson and Sommerfeld (1) for animals of 190 grams as 0.0125 grams. The rate of injection was in all our experiments 1 cc. per minute. Our results were as follows:

Animals weighing 1) 210, 2) 205, 3) 210, 4) 210, 5) 180, 6) 250, 7) 205 and 8) 205 grams received 2 cc. of a 1 per cent KCl solution intravenously. The first six animals died while the last

two survived without showing any symptoms. A smaller dose of KCl sometimes may prove fatal to animals of about 200 grams, just as no. 6 weighing 250 grams succumbed to a dose of 2 cc., while animals of such weight usually show little effect from that amount of KCl. All animals dying after an injection were subjected to a post mortem examination and when lesions due to disease were found, the experiments were discarded. Death appears to be due to paralysis of the respiratory centers. Very frequently the heart was found beating a considerable time after cessation of respiration.

When the injection was made with a solution containing 5 per cent NaCl in addition to the 1 per cent KCl, the result was this: Two animals of 190 and 155 grams received 2 cc.; two animals of 165 and 205 grams received 3 cc.; an animal of 195 grams 4 cc.; two animals of 195 and 200 grams 5 cc. and an animal of 205 grams 6 cc. All survived showing hardly any effect from the injection. It is evident that the addition of NaCl to a KCl solution depresses the toxicity of the latter.

Two animals weighing 193 and 185 grams received 4 cc. of a solution containing 3 per cent NaCl and 1 per cent KCl and both survived. A number of experiments were made with mixtures containing 1 or 2 per cent NaCl and 1 per cent KCl. While in both sets some animals survived, these experiments have to be discarded and for this reason: The experiments were not always accompanied by controls injected with a 1 per cent KCl solution, and in the last sets the controls survived also (nos. 7 and 8). All the other experiments cited here were made on the same day on which a control had succumbed to 2 cc. of a 1 per cent KCl solution, and the animals were of the same batch obtained from the dealer, having been kept several days before use under the same conditions. Such precautions proved by no means superfluous, when on account of the survival of the above mentioned controls, we continued our experiments with a 1.5 per cent solution of KCl. Of thirty guinea-pigs weighing 190 to 230 grams, seven survived an injection of 1.5 to 2 cc. of a 1.5 per cent KCl solution. Of these an animal of 215 grams had received 1.5 cc., two animals of 225 and 222 grams 2 cc. and four more animals

weighing 195, 198, 200, and 205 grams survived a dose of 2 cc. These last four animals were of the same batch and distinguished themselves from the other surviving animals, in that they did not show the slightest symptoms, while the other three showed a marked effect on the respiration and had severe convulsions. The rest of the animals of this batch were kept exclusively on carrots for three days while their previous diet had included bread. That is, the animals were now kept on a diet poor in NaCl. After this time two animals of 193 and 190 grams promptly succumbed to 2 cc. 1.5 per cent KCl. It is not certain though, that the change in diet alone entered into consideration, making the animals susceptible to the potassium salt. It is true, they looked well and were lively but they had not gained in weight. That a change of diet can influence the toxicity of various substances, such as of various fatty oils and of sodium tartrate has been demonstrated lately by Salant in coöperation with Bengis (2) and Swanson (3).

Twelve animals weighing from 190 to 212 grams received 2 cc. of a mixture containing 5 per cent NaCl and 1.5 per cent KCl. Of these twelve animals, three which weighed 190, 197, and 208 grams died. Of the remaining nine animals, six had no or only slight symptoms, such as irregularity of respiration and very slight convulsive seizures. These animals weighed 210, 222, 190, 195, 207, and 190 grams. Three animals of 198, 198, and 200 grams had more pronounced convulsions and the respiration was more affected, in one case the respiration became gasping.

This unequal effect recalls an observation of Loeb and Wasteneys (4) that some fundulus could live in a KCl solution when only a trace of CaCl_2 was added, while others required CaCl_2 .

It would have been better if only animals of known parentage and raised under the same conditions could have been used for our experiments but that was impossible.

A guinea-pig of 210 grams survived an injection of 3 cc. of the solution, showing very little effect. Four animals of 190, 195, 205, and 205 grams received 4 cc. One died, the other three were little affected. Two animals of 205 and 208 grams received

5 cc., both had some convulsions, though not severe, and recovered rapidly.

Next we employed a solution containing 3 per cent NaCl and 1.5 per cent KCl. Five animals of 194 to 198 grams received 2 cc. Two died, of the three surviving ones, one had a short cessation of respiration, one some convulsions and one showed little effect, that is, the symptoms confined themselves to some irregularity of the respiration. Two animals weighing 196 and 198 grams survived a dose of 4 cc., one without symptoms, in the other the respiration stopped for a short time, returning with gasping and there were quite severe convulsions.

In another series of experiments the injections were made with a solution containing 10 per cent NaCl and 1.5 per cent KCl. Two animals (186 and 193 grams) received 2 cc. of this solution, and in both a short cessation of respiration occurred but no convulsions. One animal of 182 grams died. Two animals of 212 grams received 4 cc. Both had convulsions and irregularities of the respiration, but recovered quickly. The increase in the concentration of the NaCl from 5 to 10 per cent did not give any better results. Controls with 10 per cent NaCl gave the following results: An animal of 212 grams received 4 cc. in three and one-half minutes and showed some effect. Two animals of 225 and 230 grams received 5 cc. in four minutes without showing any definite symptoms, while an animal of 220 grams died from a dose of 4.5 cc. administered in five minutes.

Some interesting results were obtained on injection of 10 per cent NaCl, which deserve brief notice. A number of animals (twenty-two) varying in weight from 219 to 340 grams received from 2 to 10 cc. of 10 per cent NaCl solution. The lenses of the eyes became cloudy in from ten to twenty minutes after the injection. When the animals survived, this cloudiness disappeared in the course of three to four hours. In some cases this cloudiness increased and a precipitate seemed to form in the lenses, arranged in a network. Such a lense excised and placed in water clears up entirely while a normal lense placed in a strong NaCl solution presents the same changes as seen in the living animal. The injection of strong NaCl solutions in the frog is known to be followed by a very visible effect on the red cor-

puscles. At no time after the injection of the 10 per cent NaCl solution could we observe any change in the form of the red blood corpuscles. Neither was there any very marked effect on their number in observations starting fifteen minutes after the injection and continued for several days.

A few experiments were made with a 2 per cent KCl solution. Here larger animals were used. Two animals of 245 and 269 grams survived 0.5 cc. of a 2 per cent KCl solution readily. Four animals of 260 to 275 grams succumbed to a dose of 1 and 1.5 cc. Two animals of 265 and 275 grams survived the injection of 2 cc. of a solution containing 5 per cent NaCl and 2 per cent KCl. Some animals of 250 to 255 grams received more than 2 cc. All of these died. The results of all these experiments show a marked diminution of the toxicity of KCl in the presence of NaCl, when both these substances are contained in the same solution.

The same effect became evident on a different arrangement of the experiment, where the animals received first an intravenous injection of a NaCl solution followed within a short period of time by an injection of the 1.5 per cent KCl solution either in the same vein or in the vein of the other side. In most of the experiments 5 cc. of a 5 per cent NaCl solution were given, the injection lasting four minutes. Only in the last two experiments 4 cc. of a 10 per cent NaCl solution was given at the rate of 1 cc. per minute. The results were:

WEIGHT	1.5 PER CENT KCl	TIME ELAPSED BETWEEN INJECTIONS	REMARKS
<i>grams</i>	<i>cc.</i>	<i>seconds</i>	
191	2.0	20	No symptoms
193	2.0	30	No symptoms
187	2.0	40	No symptoms
194	2.0	105	Short cessation of respiration. Some convulsions
212	2.0	15	Little sick
185	3.0	30	Twitching excitement. Little sick
210	3.0	30	Died
192	3.0	50	No symptoms
208	3.0	25	Short cessation of respiration. Some convulsions
200	3.0	35	Twitching. Little effect
212	3.0	30	Twitchings, irregular respiration. Little sick

Using such small animals the possibility had to be considered that intravenous injection of 5 cc. fluid could produce a very marked dilution of the blood and that this dilution was really responsible for the protective action by diminishing the concentration of the subsequently injected KCl below the fatal.

A number of control experiments were made replacing the 5 per cent NaCl solution by one of 0.55 per cent. This concentration was chosen because the determination of the Cl content of the serum of a few guinea-pigs gave this figure for NaCl. In all cases the NaCl injection was followed by one of 2 cc. of a 1.5 per cent KCl solution. The results were:

WEIGHT	TIME BETWEEN THE TWO INJECTIONS	REMARKS
<i>grams</i>	<i>seconds</i>	
190	40	Twitchings, short cessation of respiration; little sick, survived
190	20	Died
203	15	Died
197	15	Died after two minutes
205	30	Died after one minute
203	30	Died
212	25	Died

These results show clearly that the injection per se of 5 cc. of fluid cannot participate to any great extent in the protective action. But a 5 per cent solution of NaCl may not only increase the volume of the blood by the amount of solution injected but in addition by attracting fluid into the vessels, thus increasing the volume of blood much more than a 0.55 per cent NaCl solution. We have before us the results of Bornstein (5) who found shortly after intravenous or intracardial injection of 1 to 1.1 cc. of a 20 to 30 per cent NaCl solution in the guinea-pig a very marked increase of blood volume measured by the change in its hemoglobin content. The hemoglobin was determined with the hemometer of Sahli. Bornstein determined the hemoglobin before and after the injection and calculated the change in volume. For instance before the injection 76 per cent hemoglobin was found; after the injection 40. If the volume of blood at the

time of the first withdrawal of blood be put as 100, the volume after the second will be $\frac{76 \times 100}{40} = 190$. Bornstein found the greatest alteration after an intracardial injection of 1 cc. 30 per cent NaCl. One minute after the injection, the blood volume had increased to 200, i.e., it had doubled. We conducted similar experiments determining the hemoglobin with the Fleishl-Miescher hemometer. The 5 cc. NaCl solutions were injected in four minutes. The results are calculated according to Bornstein.

5 per cent NaCl

WEIGHT	
<i>grams</i>	
200	Volume of blood 100 seconds after injection: 123
207	Volume of blood 100 seconds after injection: 122
205	Volume of blood 100 seconds after injection: 126

0.55 per cent NaCl

198	Volume of blood 100 seconds after injection: 124
208	Volume of blood 100 seconds after injection: 118
195	Volume of blood 100 seconds after injection: 132

These experiments do not permit the assumption that an injection of 5 cc. 5 per cent NaCl solution induces a markedly greater increase in blood volume than an injection of 5 cc. of a 0.55 per cent NaCl solution in the guinea-pig. It may be noted that no urine was passed during these experiments. In all our experiments with 5 per cent NaCl or less it happened but rarely that the guinea-pigs passed urine during the injection.

The two experiments now to be brought forward were made to test whether the presence of the mixture of salts in the circulation might produce a rapid diuresis and so remove sufficient KCl from the organism to render it harmless. An animal of 190 grams, after expressing the urine from the bladder received 2 cc. of a solution containing 5 per cent NaCl and 1.5 per cent KCl, with very little effect. Ten minutes after the injection about 0.1 cc. urine could be expressed from the bladder. Another animal of 210 gram with a dose of 3 cc. of the same solu-

tion also showed little effect and yielded after ten minutes about 0.2 cc. of urine. Two of the controls succumbed promptly to 2 cc. of 1.5 per cent KCl. A time of ten minutes may be regarded as ample for our purposes, for none of our animals ever died later than five minutes after a fatal dose of KCl. If an animal survived five minutes, it recovered. A hastening, therefore, of the KCl excretion cannot be accepted as a factor in the protection offered an animal by NaCl.

In the experiments reported thus far we employed salts possessing a common ion. Now the KCl was replaced by K_2SO_4 . A solution of K_2SO_4 , corresponding in its K content to a 1 per cent KCl solution seems to be less toxic than the latter. Thus of seven animals (weighing 230, 185, 195, 180, 209, 198, 202 grams), none succumbed to 2 cc. of an approximately 1.18 per cent K_2SO_4 solution. Two animals, weighing each 215 grams survived 3 cc. and of two others, weighing 218 and 219 grams, one survived 4 cc. With a 1.75 per cent solution of K_2SO_4 , corresponding about to a 1.5 per cent KCl solution with reference to its K content, the results were as with the latter solution. Of ten animals, weighing 197 to 216 grams, three survived 1.5 or 2 cc., one of the latter animals belonged to the group described above as refractory to a 1.5 per cent KCl solution. With a solution containing 5 per cent NaCl and 1.75 per cent K_2SO_4 the results were as follows:

WEIGHT	CUBIC CENTI- METERS OF MIXTURE	REMARKS
<i>grams</i>		
192	2.0	Hardly any symptoms
197	2.0	Hardly any symptoms
203	3.0	Died
210	4.0	No symptoms
215	4.0	No symptoms

A solution of 2.3 per cent K_2SO_4 (about equal to 2 per cent KCl) proved fatal three times out of four in a dose of 0.5 cc. to animals weighing 205 to 237 grams and a dose of 1 cc. killed four animals from 210 to 250 grams.

The few experiments with a mixture containing 5 per cent NaCl and 2.3 per cent K_2SO_4 showed the following:

WEIGHT	CUBIC CENTI- METERS INJECTED	REMARKS
<i>grams</i>		
215	1.0	Died
215	1.0	Cessation of respiration, gasping, survived
205	1.0	Cessation of respiration, gasping, survived
245	2.0	Short cessation of respiration, convulsions. Survived

When the injection of 2 cc. 1.75 per cent K_2SO_4 was preceded by 5 cc. of a 5 per cent or 0.55 per cent NaCl solution the following results were obtained:

5 per cent NaCl

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
<i>grams</i>	<i>seconds</i>	
197	15	Some twitching
198	90	Some twitching
195	15	Hardly any symptoms
187	35	Hardly any symptoms

0.55 per cent NaCl

196	20	Died
190	20	Died
190	20	Died

The results of the experiments with NaCl and K_2SO_4 closely resemble those with NaCl and KCl, in as much as a very marked protective influence of the NaCl becomes evident. In another series of experiments the NaCl was replaced by sodium salts. We used a Na_2SO_4 solution about equal in concentration to a 5 per cent NaCl solution with reference to its Na content. The potassium salt was KCl. A few experiments were made with a mixture containing 1 per cent KCl. Two animals of 190 and 170 grams survived an injection of 4 cc. with hardly any symptoms. Ten animals received 2 cc. of a mixture containing 6 per cent of the dry Na_2SO_4 and 1.5 per cent KCl. The weight of these

animals was: 1) 196, 2) 196, 3) 197, 4) 190, 5) 190, 6) 205, 7) 187, 8) 190, 9) 185, 10) 185. Of these animals nos. 1-5 survived, none showing much effect, while nos. 6-10 died. No. 8 died after four minutes. Some experiments were also made following an injection of 5 cc. of the Na_2SO_4 by one of 2 cc. 1.5 per cent KCl. This seemed feasible from our first experiments where two control animals of 195 and 205 grams received 5 cc. of the Na_2SO_4 solution without any effect. Later two animals of 193 to 210 grams succumbed to doses of 4 and 5 cc. But three experiments were successful, that is, the animals tolerated an injection of 5 cc. Na_2SO_4 solution without any apparent effect and the KCl could be given immediately after

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
<i>grams</i>	<i>seconds</i>	
203	15	Not sick
214	15	Irregular respiration, some convulsions, little sick
215	20	Died

We may say that sodium sulphate can exercise a protective influence against KCl, although it does not seem to be as effective as NaCl.

Some trials were made with sodium acetate. The solution made from the salt containing 3 molecules of water of crystallization was about 11.7 per cent, corresponding about to a 5 per cent NaCl solution with reference to the Na. The arrangement of the experiment was thus: Two animals of 207 and 212 grams received 2 cc. of the sodium acetate solution without any effect. An animal of 215 grams promptly died on the injection of 2 cc. 1.5 per cent KCl. Three animals weighing 205, 207, and 212 grams received 2 cc. of a solution containing sodium acetate in the strength given and 1.5 per cent KCl. These three animals died, while two others of 210 and 212 grams weight survived the injection of 2 cc. of a mixture containing 5 per cent NaCl and 1.5 per cent KCl, without any visible effect. It seems evident even from this small number of experiments that sodium acetate cannot replace the sodium chloride in its protective action against KCl.

Desiring to test whether NaCl protected equally well against the toxic effects of other than potassium salts, the latter were replaced by ammonium chloride. First the fatal dose of this substance had to be determined. These experiments were carried out with a 2 per cent NH_4Cl solution. Animals, weighing 200, 210, and 220 grams showed little effect from a dose of 1.5 cc.

Ten animals varying in weight from 190 to 230 grams received 2 cc. and only one animal weighing 220 grams recovered from a rather severe attack of intoxication, lying on its side with a gasping respiration for more than thirty minutes. The most striking lesions of a fatal dose of NH_4Cl on intravenous injection in the guinea-pig are edema of the lungs, and the presence in the lungs of multiple hemorrhages of various sizes, but as a rule rather small.

Seven animals weighing from 190 to 228 grams received 2 cc. of a mixture containing 5 per cent NaCl and 2 per cent NH_4Cl , one of 180 grams received 1.8 cc. Of these eight animals five died and the three which recovered passed through a severe attack of intoxication. The weights of these three animals were 200, 200, and 228 grams.

When an injection of 5 cc. of a 5 per cent or 0.55 per cent NaCl preceded one of 2 cc. of a 2 per cent NH_4Cl the following occurred:

5 per cent NaCl

WEIGHT	TIME BETWEEN THE INJECTIONS	REMARKS
<i>grams</i>	<i>seconds</i>	
205	20	Sick—recovered
202	20	Sick—recovered
197	20	Sick—recovered
202	30	Sick—recovered

0.55 per cent NaCl

219	25	Sick—recovered
192	25	Died
212	25	Died
207	20	Died

Three animals which survived after receiving the preliminary injection of 5 per cent NaCl were sick, lying on their side fifteen to twenty minutes and showing some hypersensitiveness on stimulation and some dyspnoea. The surviving animal of the 0.55 per cent NaCl series behaved in the same manner. The result of the experiments indicates the possibility of some protective action.

Under the conditions of our experiments, sodium salts, such as sodium chloride or sodium sulfate are capable of neutralizing the toxic action of potassium salts, such as potassium chlorid or potassium sulfate, on intravenous injection in a warm blooded animal. A number of observations are recorded in which the toxic action of the potassium chlorid has been counteracted by sodium chlorid. Of these we may recall the experiments of Hyde (6) on *Raia erinacea* and *binoculata*, where an injection of NaCl following one of KCl quickly counteracted the effect of the latter. Garrey (7) demonstrated a detoxifying effect of NaCl on KCl in the fresh water fish *Notropis blennius*. The most comprehensive data on antagonist salt action, we owe to J. Loeb (8) and his co-workers. Beginning with the striking experiments of the antagonistic action of NaCl on KCl intoxication of fundulus and vice versa, J. Loeb enlarged the scope of our knowledge on antagonistic salt action, utilizing particularly the very convenient faculty of the fundulus egg to tolerate large variations in the osmotic pressure of its surroundings. These experiments put the detoxifying action of NaCl on KCl in place with the other antagonistic salt actions.

As examples of antagonistic salt action in the warm blooded animal, we may cite the detoxifying effect of KCl on NaCl observed by Joseph and Meltzer (9) and that of $MgSO_4$ on sodium oxalate, observed by Gates and Meltzer (10).

The attempt at an explanation of the results of our experiments has to take into consideration a number of factors. When the solutions of the salts are mixed before being injected, complicated reactions occur in the solution (see Smith and Ball (11)), which will exercise some influence on the result of the injection. Such reactions may also participate in the result when the injection of

one salt follows that of the other. The action of any substance is dependent on its reaching the tissues it attacks in sufficient concentration for a sufficient length of time. The failure of the KCl to kill in our experiments may be due to factors preventing it from reaching its destination in sufficient concentration for a sufficient length of time. The toxic action of KCl in the guinea pig seems to be directed chiefly against the central control of the respiration. It may be prevented from arriving there in the necessary concentration in various ways. A somewhat remote possibility exists in the influence one salt might perhaps exercise on the combination of the other with a constituent of the blood. Such a possibility is indicated by the observation of Oryng and Pauli (12), that a neutral salt (NaNO_3) causes a slight increase in the combining power of serum for Cl from KCl. The possibility of a hastening of the excretion of KCl by NaCl was excluded in our experiments. Another factor is to be thought of in the increase of the blood volume brought about by the injection of the hypertonic salt solutions, so that the KCl would no longer attain the necessary concentration. The experiments in which the change of blood volume was determined after the injection of 5 cc. of a 5 per cent NaCl showed a definite increase but not more than after the injection of 5 cc. of a 0.55 per cent NaCl; while the protective action of the 5 per cent NaCl solution was pronounced in contrast to that of the 0.55 per cent NaCl solution. The method of the estimation of the changes in blood volume by means of the hemoglobin determination may be none too accurate but large differences could hardly have been missed. While the increase in blood volume may be a contributory factor in the protective action afforded by the NaCl, we cannot explain our results on this basis alone. In other experiments the increase of blood volume may be of greater importance. Five guinea pigs received 2 cc. of a solution containing 50 per cent glucose and 1.5 per cent KCl. The weight of these animals was 190, 195, 195, 207, and 207 grams. All survived, showing hardly any effect. An injection of 2 cc. 50 per cent glucose is readily tolerated by guinea-pigs of such weight. In several instances the change of blood volume after the injection of 2 cc. 50 per cent glucose was

determined in the same manner as in the experiments with NaCl. The results were:

WEIGHT	
<i>grams</i>	
196	Increase of blood volume 35 seconds after injection to 142
198	Increase of blood volume 35 seconds after injection to 139
200	Increase of blood volume 35 seconds after injection to 166 $\frac{2}{3}$
200	Increase of blood volume 35 seconds after injection to 143

In these experiments the vessels of the ears were standing out very full in marked contrast to their appearance even after injections of 5 cc. 5 per cent NaCl solutions. In these cases there is a marked increase in blood volume, i.e., fluid enters the vessels rapidly as in the experiments of Bornstein with the more concentrated NaCl solutions. The rapid dilution of the blood in such cases will be of great importance provided the KCl does not leave the vessels as rapidly as the fluid enters.

In the case of NaCl there are some data indicating that the NaCl may perhaps not leave the vessels as rapidly as water enters. Dr. Eyster (13) was kind enough to tell us of some experiments in which he injected saturated NaCl solutions intravenously in dogs, determining the lowering of the freezing point. At first the blood was hypertonic but within five minutes the freezing point was normal. Eyster interprets these results as meaning, that water entering the vessels more rapidly than the salt can leave, raises the freezing point of the blood to its normal level. Nowaczek (14) injected large amounts of 10 per cent NaCl intravenously in animals and states that the concentration of NaCl in the blood was never greater than in the urine, indeed his figures for the concentration in urine exceed those for the blood quite markedly. This would speak in favor of a rapid exit of NaCl from the vessels. His tests were made in fifteen minute intervals, and from the data at our disposal, his observations do not include the happenings within the first minutes, the time which concerns us here.

The permeability of the vessel walls need not be the same for all salts and so it will be necessary to determine it separately for the

different salts. On the other side, even if the KCl should leave the vessels with the same velocity with which fluid enters, another mode of its deviation from the central nervous system is conceivable. It is known that muscles and skin are the tissues most important for the storage of water (see Engels: *Die Bedeutung der Gewebe als Wasserdepots*. Arch. f. expt. Pathol. u. Pharmacol., 1904, no. li, p. 346) and the muscles can give up considerable fluid on withholding water (Straub: *Ueber den Einfluss der Wasserentziehung auf den Stoffwechsel und Kreislauf*. Zeitschr. f. Biolog., 1899, vol. 38, p. 537). Should the tissues give up water to the blood with varying degree of ease, the KCl might chiefly go to those organs giving up their water more readily and thus be prevented from displaying its toxic action.

The absence of data precludes any further discussion of such possibilities as pointed out.

It is probable that in our experiments with NaCl and KCl the latter arrived at the central nervous system in sufficient concentration to display its toxic effect, particularly with the injection of the solution containing 5 per cent NaCl and 2 per cent KCl. Under such conditions, the fundamental experiments of J. Loeb (8) on the fundulus egg give a clew as to mechanisms by which such "antagonistic salt actions" are brought about. A comparison of our results with those of Loeb cannot be attempted at present, for we have no way of knowing the concentrations in which the salts arrive at the regions in the central nervous system where the antagonistic salt action is exercised. Besides, the differences of the routes they have to travel before reaching this destination must play a rôle, as we have pointed out above. Finally, the difference between the cells to be acted upon is too great for comparison.

We must not forget that an antagonism between NaCl and KCl has been noted by Zoethut (15) working with the gastrocnemius of the frog, where the application of NaCl previous to or simultaneous with the application of KCl hinders the development of the potassium contraction. In Hoeber's (16) experiments, Na inhibited the toxic effect of K on the skeletal muscle.

Of interest is the statement of Clowes (17):

It has been found possible, using drop systems containing a large amount of oleic acid in conjunction with Na oleate, to produce similar somewhat feeble antagonistic effects between KCl and NaCl, although usually in a NaOH system, KCl functions like NaCl as an antagonist for CaCl_2 .

The recent observations of Loeb (18) on the formation of ionized gelatin on treatment with NaCl contain much promise for the better understanding of antagonistic salt actions.

SUMMARY

Guinea-pigs survive the intravenous injection of otherwise fatal doses of KCl and K_2SO_4 if they are administered in mixtures with NaCl.

The same effect is noted when Na_2SO_4 is added to KCl solution. The effect is, however, not quite so marked. In these experiments the concentration of the Na_2SO_4 was equal to that of the 5 per cent NaCl solution with reference to its Na content.

An equal concentration of sodium acetate does not afford any protection against the KCl intoxication.

The protection offered by NaCl against NH_4Cl is somewhat doubtful, at any rate it is much less marked than against KCl.

The presence of 50 per cent glucose in the KCl solution protects the animals very markedly.

A usual fatal dose of KCl or K_2SO_4 injected soon after an injection of 5 per cent NaCl, is tolerated, while a previous injection of 0.55 per cent NaCl does not protect, or only very rarely.

Where a previous injection of Na_2SO_4 is tolerated it may also protect against KCl.

The previous injection of NaCl seems to protect against NH_4Cl .

Several possibilities which may contribute to such protective actions, as are here described, are pointed out.

The intravenous injection of 10 per cent NaCl solution in proper dosage produces a clouding of the crystalline lens. This cloudiness appears within about twenty minutes and disappears in the course of about four hours.

- (1) AARONSON AND SOMMERFELD: *Deutsch. med. Wochschr.*, xxxix, i, 1913, 455.
- (2) SALANT AND BENGIS: *Jour of Pharmacol. and Exp. Therap.*, ix, 1917, 529.
- (3) SALANT AND SWANSON: *Jour. of Pharmacol. and Exp. Therap.*, xi, 1918, 27.
- (4) LOEB, J., AND WASTENEYS: *Biochem Zeitschr.*, xxxii, 1911, 308.
- (5) BORNSTEIN: *Zeitschr. f. Immunitätsforschung*, xiv, 1914, 607.
- (6) HYDE: *American Jour. of Physiol.*, xxiii, 1908-09, 201.
- (7) GARREY: *American Jour. of Physiol.*, xxix, 1916, 313.
- (8) LOEB, J., and WASTENEYS: *Biochem. Zeitschr.*, xxxi, 1911, 450, xxxiii, 1911, 480.
LOEB: *Biochem. Zeitschr.*, xxxiii, 1911, 480.
LOEB: *Biochem. Zeitschr.*, xxxvi, 1911, 275.
LOEB AND WASTENEYS: *Biochem. Zeitschr.*, xxxix, 1912, 185.
LOEB: *Biochem. Zeitschr.*, xxxix, 1912, 194.
LOEB: *Biochem. Zeitschr.*, xliii, 1912, 181.
LOEB: *Jour. of Biolog. Chem.*, xix, 1914, 431.
LOEB AND WASTENEYS: *Jour. of Biolog. Chem.*, xxi, 1915, 223.
LOEB AND CATTELL: *Jour. of Biolog. Chem.*, xxiii, 1915, 41.
LOEB: *Jour. of Biolog. Chem.*, xxvii, 1916, 339, 353, 363.
LOEB: *Jour. of Biolog. Chem.*, xxviii, 1916, 175.
- (9) JOSEPH AND MELTZER: *Proceedings Soc. for Exper. Biol. and Med.*, viii, 1910, 14.
- (10) GATES AND MELTZER: *Jour. of Pharmacol. and Experim. Therap.*, ix, 1917, 353.
- (11) SMITH AND BALL: *Jour. of Amer. Chem. Soc.*, xxxix, 1917, 179.
- (12) ORYNG AND PAULI: *Biochem. Zeitschr.*, lxx, 1915, 368.
- (13) EYSTER, A. E.: Verbal communication.
- (14) NOWACZEK: See Lindemann: *Die Funktionen der Niere. Ergebnisse der Physiologie*, xiv, 1914, 628.
- (15) ZOETHUT: *Amer. Jour. of Physiol.*, xxiii, 1908-9, 374.
- (16) HOEBER: See *Chem. Abstracts*, xii, 1918, 495.
- (17) CLOWES: *Jour. of Physical Chem.*, xx, 1916, 434.
- (18) J. LOEB: *Jour. of Biol. Chem.*, xxxi, 1917, 343.

THE ACRIDITY OF SOME PLANTS DUE TO A MECHANICAL ACTION

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It is quite generally known that cowhage, which is the hairs removed from the pods of *Mucuna pruriens*, when placed in contact with the skin, causes an intolerable itching which is greatly increased by rubbing. The irritation depends wholly upon the penetration of the hairs into the skin which act as a mechanical irritant.

We have a number of plants, some of which are employed for their therapeutic action, which contain an irritant body usually spoken of as an acrid principle.

The attention of one of us (Brown) having been called to an alarming condition brought about by the eating of a portion of an Indian turnip (*Arisaema triphyllum*), by a small child, it was thought it might be of interest to determine the nature of the acrid principle.

The National Standard Dispensatory states that "in the fresh state arum has an acrid, burning taste and acts as a severe irritant to mucous membranes with which it comes in contact.

"If given internally, it causes severe gastro-enteritis. The acrid principle is dissipated by drying and by heat."

The United States Dispensatory gives practically the same information and further states that it contains a neutral volatile acrid principle soluble in ether and also saponin.

The information given in both of these works is evidently based upon the work done by some of the earlier investigators who arrived at a conclusion that a volatile acrid principle must be present, since they were unable to isolate any substance having the characteristic action of the fresh corm.

Jones (1) states that all attempts to isolate the principle upon which the acidity depends have proved unsatisfactory. After making a complete analysis of the corm, he concludes that it contains a peculiar, pungent, acrid principle which he was unable to isolate.

Enz (2) reports having extracted a volatile acrid principle where ether was employed as the solvent. He also claims to have found saponin as one of the constituents.

In 1891, Weber (3) undertook to determine the nature of the acrid principle acting on the suggestion that the acidity was due to the raphides of calcium oxalate which are abundant in the plant and likewise in other plants producing a like acrid sensation. He selected four plants containing raphides, two of which, the Indian turnip and *Callocassia*, were highly acrid, and two, the *Fuchsia* and *Tradescantia*, were perfectly bland to the taste.

From the *Callocassia* he was able to obtain the crystals in a pure state, while from the *arum* they were more or less contaminated with starch. He found it impossible to separate the crystals from the *Tradescantia* and *Fuchsia* owing to the large amount of mucilage present, but found the crystals in all cases to be calcium oxalate. He also found that the ether extract was acrid when applied to the tongue, thus confirming the observation of Enz, and seemed to corroborate the assumption that the acrid principle was soluble in ether.

He later found that the ether extract contained an abundance of the crystals. After filtering through a Munktell filter the ether solution was entirely free from raphides and was no longer acrid. He concludes from this experiment that the acidity of the *Callocassia* and *arum* is due to the raphides of calcium oxalate only. In the case of the *Tradescantia* and *Fuchsia* which contained an abundance of the crystals and is not acrid, he was unable to separate the crystals from the large amount of mucilage present and explains their non-acridity on the grounds that the crystals are surrounded with, and embedded in an insoluble mucilage, which prevents their free movement into the tissues of the tongue and mouth when the plant is tasted. He explains

the loss of acidity in the arum on the assumption that the starch acts in a similar manner to mucilage. Other investigators have denied this explanation and ascribe the acidity to saponin.

What appeared interesting to us was how either of these explanations could account for the loss of acidity after the corm had been dried or boiled.

The calcium oxalate crystals are not lost on drying and the same may be said of saponin.

Naturally one might conclude that a volatile acrid principle was present, which was lost in the process of either drying or on boiling.

In order to determine whether this might be the case, several fresh corms of the arum were placed in a boiling flask provided with a long condenser. Heat was applied to the flask and the distillate collected. In order to exclude the possibility of a volatile principle escaping before the distillate started to come over, the tongue was held over the end of the condenser at frequent intervals without experiencing any sensation. The tongue was also applied to the end of the condenser as the first drops of the distillate came over and at frequent intervals during a period of one and one-half hours, and at no time was there any evidence of an acrid principle.

A large amount of the distillate held in the mouth was ineffective as regards irritation, and even the swallowing of some of the liquid gave no effect. This result rather tends to exclude the possibility of a volatile acrid principle being present although it is conceivable that such a body might be present and was destroyed by the heat, but this was thought improbable.

The boiled corms and the water in which they were boiled was also free from acidity.

This result led to an investigation of the mechanical theory, namely, the raphides of calcium oxalate since the saponin explanation appeared the least plausible from the fact that the irritation produced by saponin is not comparable with that produced by arum.

Under the microscope, scrapings of the fresh corm show an abundance of acicular crystals along with a large number of starch granules. The boiled corm shows the crystals still present but they are broken in pieces from one-fourth to one-half of their original length. A preparation of the air dried corm prepared in the same manner after thoroughly soaking in water to soften the tissue was also devoid of acidity and the crystals were broken as in the boiled corm. This offered an explanation for the loss of acidity, and at the same time gave strong evidence that the crystals were responsible for the action.

In order to determine whether this was a true mechanical action, a thin slice of the corm was carefully laid on the tongue of each of the authors and allowed to remain for five minutes after which time it was very carefully removed so as not to produce any friction on the surface of the tongue. There was no evidence of acidity experienced by either of us. The same slice of the corm was then gently rubbed over the tip of the tongue which produced the characteristic sensation. This showed quite conclusively that the crystals were responsible for the irritation and that they penetrated the tissues only when brought in contact through friction which tended to cause their penetration.

To verify this, some of the grated corm was vigorously rubbed between the palmar surfaces of the hands for a short period of time. The result was more than had been expected. A sensation of tingling of a more or less painful character was experienced which was not entirely gone at the end of three days. The hands also felt swollen and stiffened and as though they were full of needles but there was no visible evidence of irritation.

This experiment gave conclusive evidence that the action was of a mechanical nature and may be placed in the same class as *mucuna* previously mentioned.

A number of other plants known to contain raphides were also studied in the same manner as the *arum* in order to determine whether acidity was due to the presence of these crystals. Two of the common calladiums, *Callocassia callocassia* and *Callocassia esculenta*, were obtained in the fresh condition, the former being the most fiery of any plant examined.

The crystals are about the same length as those found in the arum and somewhat less in diameter. A slice of the rhizome like root was boiled for three hours after which it was rubbed on the tongue. Very little tingling sensation was produced. Thinking perhaps the crystals on the surface might have been washed out during the boiling process, a thin slice was removed from the surface leaving a fresh surface which was found to have retained all of its original fiery taste.

The water in which it had been boiled was centrifuged and the precipitate examined for crystals. As expected they were found in abundance thus verifying the supposition that the crystals on the surface had been washed off rather than that it had lost its acidity due to heat.

The crystals are not broken in either the dried or boiled root, therefore, the acidity is retained.

Dracontium, skunk-cabbage. The dried rhizome and roots of *Spathyema foetida*. The National Standard Dispensatory states that "The active constituent appears to be the volatile principle which is dissipated by heating and disappears upon keeping."

None of the fresh rhizome was available but the dried rhizome which had been kept for at least two years gave a sensation when rubbed on the tongue.

After boiling for one hour and again rubbing on the tongue the acrid sensation was present but not to the same degree as that given by the arum.

Microscopic examination shows the presence of acicular crystals.

Phytolacca is another drug which the Dispensatory describes as containing an acrid substance very similar to saponin. Microscopic examination shows the presence of acicular crystals in large numbers which are shorter and thicker than those found in the arum or callocassia. The fresh, dried and boiled root gave a tingling sensation to the tongue but not nearly as marked as the arum. I am informed that men employed in drug milling plants experience severe and distressing symptoms when this drug is ground. The mucous membranes are extensively involved in some cases causing total incapacity for work for a period of several days. That the condition is in all proba-

bility due to the crystals is shown by the fact that the sputum is loaded with the crystals and the bronchial symptoms clear up as the crystals disappear.

The degree of so-called acidity is governed by the physical character of the crystals and the character of the plant tissues in which they are embedded, those plants containing the long, very slender crystals being much more acrid than those where the crystals are shorter and thicker.

As for the loss of acidity in the arum after drying or boiling this can be explained by the fact that the crystals are broken up in the process. A further explanation as to why the crystals in the arum should be broken in either of these processes and not in the other plants, may be the fact that the crystals in the arum are not inclined to occur in bundles as in the others and in the shrinking due to drying they become broken by unequal pressure along their body.

When the arum is boiled, the swelling of the starch granules, which constitute the greater part of the corm, acting in a like manner causes the crystals to break. The other plants examined are composed largely of fibrous tissue and the crystals are in bundles thus capable of withstanding unequal strain which would tend to cause their fracture.

Some of these plants possibly contain a small amount of saponin and may impart a mild degree of acidity but this is overshadowed by the fiery and painful sensation produced by the penetration of the raphides of calcium oxalate which act as a mechanical irritant.

REFERENCES

- (1) JONES: *Am. Jour. Pharmacy*, July, 1843.
- (2) ENZ: *Am. Jour. Pharmacy*, xxxi, 351.
- (3) WEBER: *Jour. Am. Chem. Society*, 1891, p. 215.

I. TONUS WAVES FROM THE SINO-AURICULAR MUSCLE PREPARATION OF THE TERRAPIN AS AFFECTED BY ADRENALIN

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In 1887 Fano (1) demonstrated tonus or rhythmical oscillatory waves, independent of auricular contractions in the auricles of the terrapin, *Emys europaea*. These waves, he reports, were seldom seen in the ventricles. Howell (2), on the contrary, remarks that tonus waves are frequently seen in the ventricles and ventricular strips of the terrapin's heart. Gaskell (3) studied the heart of the land tortoise but never observed tonus waves similar to those obtained by Fano. In the three experiments performed, Fano found that the waves disappeared when the temperature was increased to 32°, 36°, and 40°C., respectively. Fano and Sciolla (4), working upon intact hearts in the same species of animals, corroborated Fano's earlier work, namely, that heat causes a disappearance of the waves and an increase in the force of the contractions. They observed that the waves persisted and were even increased during vagus stimulation, although the contractions of the auricles were entirely inhibited.

Experiments were performed by these authors demonstrating the action of certain drugs upon the tonus waves. They found that muscarine sulphate markedly increased them and caused a disappearance of the contractions, as did vagus stimulation. They also reported that in those animals in which the tonus waves were not present muscarine sulphate caused them to appear. A saturated solution of digitalin was found to decrease the height of contraction and tonus waves, but to increase the general tonus. On the contrary, the following drugs—alkaloid of atro-

pine and the salt, atropine sulphate, 1 per cent alkaloid of nicotine, veratrine, ellebroine, and a saturated solution of caffeine (alkaloid)—caused a disappearance of the tonus waves. In the nicotinized heart muscarine could not bring back the tonus waves nor was it capable of paralyzing the contractions.

Fano and Fayod (5) showed that an increase in temperature caused a disappearance of, whereas vagus stimulation caused an increase in, the rhythmical oscillations.

With the auricles in situ Bottazzi (6) observed tonus oscillation in the following animals, *Bufo viridis*, *Bufo vulgaris*, *Rana esculanta*, and *Emys europaea*. Chloroform and potassium chloride caused a decrease in the contractions and the tonus oscillations. In the auricles of *Lacerta viridis*, of *Tropidonotus natrix*, of *Anguilla vulgaris*, of *Testudo graeca*, and of the *Rana temporaria* he did not succeed in making evident any oscillations similar to those seen in the other animals. From the auricles of *Tropidonotus natrix* he obtained very peculiar tracings, showing groups of three or four contractions and between these a contraction which appeared to be higher if compared with the line of the systolic apices, or not as low as the others if compared with the diastolic line. He attributed the systolic contractions to the anisotropic substance and the slower oscillatory waves to the undifferentiated sarcoplasm. The presence of smooth muscle fibres in the terrapin auricles has been demonstrated histologically by Rosenzweig (7), and Bottazzi and Ganfini (8). To this fact they attribute the tonus waves.

Bottazzi (9) also corroborated Fano's work, that vagus stimulation markedly increases tonus waves. He observed more marked waves in those animals which were kept at a low temperature or those killed and kept in an ice chest from one to five days. An increase in tonus waves was never observed by Rosenzweig (7) in terrapin auricles upon vagus stimulation.

Recently Soraku Oinuma (10) has studied the effect of vagus and sympathetic nerve stimulation upon the oscillatory tonus waves in the *Emys europaea*, confirming Fano's and Bottazzi's observations. He obtained somewhat variable results. A marked increase in tonus waves was observed in eleven hearts upon

stimulation of the vagus nerve and in two hearts upon sympathetic nerve stimulation. A marked decrease was observed in ten hearts upon sympathetic stimulation and in one heart upon vagus stimulation.

Since sympathetic stimulation causes a disappearance or a decrease in the tonus waves, it was thought interesting to note if adrenalin, which acts upon the thoracic autonomic nervous system in other organs, would affect the tonus waves in the sino-auricular heart muscle preparation from terrapin hearts.

METHOD

Our experiments were performed upon twenty-seven terrapin of the species *Chrysemys cineria*, *Chrysemys elegans*, and *Malaclemmys lesuerii*. Six frogs of the species *Rana pipiens* were also used.

The terrapin was pithed, the plastron removed, and the heart exposed and excised, leaving a portion of the large veins attached to the auricles. The ventricles were cut from the auricles. The auricles were suspended vertically, the upper auricle connected to the aluminum wire writing lever of the heart lever, and the lower auricle fastened to a hook on an L-shaped metal rod. The up stroke on the record indicates systolic contraction, the down stroke diastolic contraction.

The entire sino-auricular muscle preparation was immersed in Ringer's solution. In some cases there was a continuous stream of oxygen bubbling through the fluid; in other experiments no oxygen was used. The adrenalin was always added to the Ringer's solution after the waves were definitely established.

RESULTS

Adrenalin caused a disappearance or a diminution in the tonus waves in all hearts experimented upon. In those experiments in which the strength of the solution was strong the waves ceased almost at once (see fig. 3). In those in which a more dilute solution was employed only a few tonus waves appeared after its ad-

dition to the Ringer's solution (see figs. 4 and 5). No oxygen was used during the making of figures 1, 2, and 3, whereas continuous oxygen was employed throughout the experiments from which figures 4 and 5 were selected.

Oinuma observed that sympathetic stimulation caused an increase in the tonus waves in some animals. We found only one

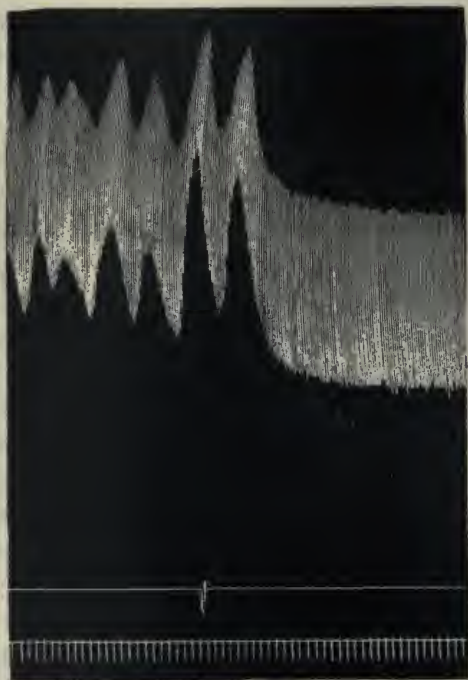


FIG. 1. ADRENALIN CHLORIDE SOLUTION, 1:154,000

In this and the following records the up stroke represents the systolic, and the down stroke the diastolic contractions of the terrapin auricles of the *Chrysemys cineria*. The upper record is that of the contracting muscles, the middle, the point of the injection of the adrenalin chloride; and the lower, time in 5 seconds.

sino-auricular muscle preparation in which adrenalin produced temporary excitation followed by complete inhibition of the tonus waves. The muscular contraction was increased. From this preparation figures 1 and 2 were made. In figure 1, two

sharp tonus waves were observed after the addition of the adrenalin (0.5 cc. of a 1: 1000 solution was added to 77 cc. of Ringer's fluid, making a dilution of 1: 154,000) at the point indicated in the record.

Immediately upon the cessation of the waves the adrenalinized Ringer's solution was removed and replaced by a fresh Ringer's solution through which oxygen was bubbling. The heart beat continued to increase in force but the tonus waves were absent for more than an hour. When they again appeared they were



FIG. 2. ADRENALIN CHLORIDE 1: 150,000 AT THE POINT INDICATED BY THE ARROW
The same auricle as that used in figure 1

much smaller than before (see fig. 2). Upon the appearance of the rhythmical oscillations the flow of oxygen through the solution was stopped. At the point indicated by the arrow in figure 2 adrenalin (0.5 cc. of a 1: 1000 solution) was added to the Ringer's solution in the beaker, making a 1: 150,000 dilution. As in figure 1 adrenalin brought about a temporary increase of the tonus waves which was followed by their complete abolition and by an increase in the force and rate of the auricular contractions.

The length of time required after an injection of adrenalin, before the reoccurrence of the waves, varied directly with the

strength of the adrenalin solution used. The addition of oxygen to the Ringer's fluid seemed to hasten the process of recovery. In three sino-auricular muscles the abolition of the waves by adrenalin was followed by small waves occurring on the systolic apices. Figure 3 is an example of this. Adrenalin in any strength did not bring about a cessation of the small waves. In figure 3 at the point indicated in the record line a sufficient amount of the drug was added to make a solution of 1:154,000. There was here, as in all other cases except the two just described, an immediate decrease of the rhythmical oscillations with an increase

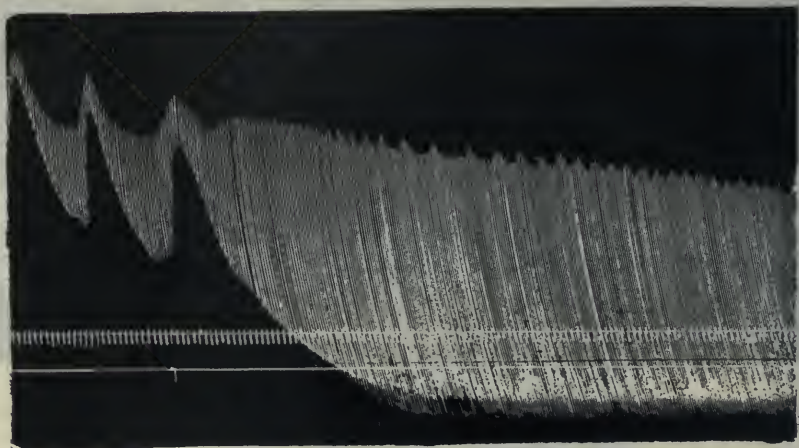


FIG. 3. ADRENALIN CHLORIDE 1:154,000

In this and the following two figures the upper record is that of the contracting auricles; the middle, time in five seconds; and the lower, the point of the injection of adrenalin.

in the force of contraction. In this preparation the rate of contraction was also increased.

The maximal dilution of adrenalin which brought about a change in oscillatory waves of the sino-auricular muscle preparation of the *Chrysemys cineria* varied with the different animals. In the majority of muscles it lay between 80,000,000 to 200,000,000.

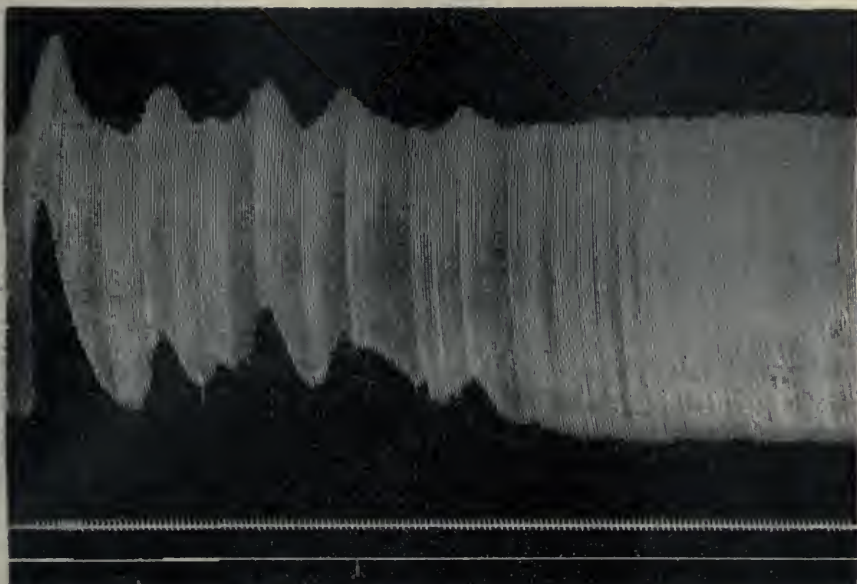


FIG. 4. EPINEPHRIN 1:80,000,000 DILUTION

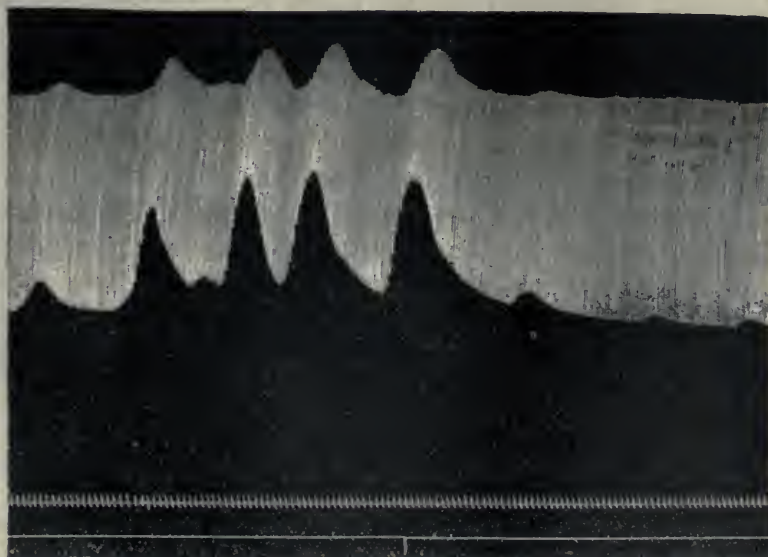


FIG. 5. ADRENALIN CHLORIDE 1:174,000,000 DILUTION

In figure 4, in which oxygen was used throughout the experiment, sufficient epinephrin was added to the Ringer's solution to make a dilution of 1: 80,000,000. Only one wave appeared after the addition of the epinephrin, but the contractions markedly increased in force and rate. A more dilute solution of adrenalin chloride (1: 174,000,000) with a continuous flow of oxygen was used in the experiment recorded in figure 5. The tonus waves immediately decreased in amplitude and finally disappeared.

In our experiments performed during May upon the terrapin of the *Chrysemys elegans* and *Malacoclemmys lesuerii* very small tonus waves were present. In many animals we were unable to obtain oscillations. We did not find, as did Bottazzi and Oinuma, that keeping the animals at a low temperature or killing them and placing them in an ice chest from one to five days affected the production of tonus waves.

Similar waves were recorded in the auricles of frogs, *Rana pipiens*.

SUMMARY

Adrenalin in dilution from 1: 150,000 to 1: 174,000,000 causes a disappearance of the tonus waves observed in the sino-auricular muscle preparation of the terrapin. It causes simultaneously an increase in the force and amplitude of contraction. In some cases adrenalin also causes an increase in the rate of contraction. After the injection of strong solutions a longer time is necessary for the reappearance of the tonus waves than with dilute solutions.

Oxygen in either case hastens the reappearance of the oscillations. This may be merely a matter of hastening the oxidation of the adrenalin.

Waves were also recorded from the auricles of frogs, *Rana pipiens*.

REFERENCES

- (1) FANO: Beiträge zur Physiologie. Festschrift zu Ludwig gewidmet. Leipzig, 1887, p. 287.
- (2) HOWELL: Textbook of physiology. Philadelphia, 1915. Sixth edition, p. 580.
- (3) GASKELL: Schäfer's Textbook of physiology. London, 1900, ii, p. 197.
- (4) FANO AND SCIOLLA: Archiv. Ital. de Biol., 1887-88, ix, p. 61.

- (5) FANO AND FAYOD: *Archiv. Ital. de Biol.*, 1887-88, ix, p. 143.
- (6) BOTTAZZI: *Journal of Physiology*, 1897, xxi, p. 1.
- (7) ROSENZWEIG: *Archiv. Physiol.*, 1903. Suppl., pp. 92 and 206.
- (8) BOTTAZZI AND GANFINI: *Zeitschr. für Allgem. Physiol.*, 1907, vi, p. 140.
- (9) BOTTAZZI: *Archiv. Ital. de Biol.*, 1900, xxxiv, p. 17; *ibid.*, 1901, xxxvi, p. 277.
- (10) SORAKU OINUMA: *Pflüger's Archiv Physiol.*, 1910, cxxxiii, p. 500.

II. TONUS WAVES IN THE TERRAPIN AURICLES AS AFFECTED BY PILOCARPINE, ATROPINE, AND ADRENALIN

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Fano and Sciolla (1) remarked that muscarine sulphate increases the tonus waves in the heart of the terrapin *Emys europaea*, and causes a disappearance of the contractions. They claim that this drug could bring about tonus waves even though they were not present at the time of the administration. Atropine alkaloid and its salt, atropine sulphate, on the contrary, caused a disappearance of the waves, if present, and counteracted those set up by muscarine.

We experimented upon the terrapin *Chrysemys cineria*, *Chrysemys elegans*, and *Malacoclemmys lesuerii* to determine whether or not pilocarpine hydrochloride (a drug identical in action on the vagus endings in the heart and also on other organs of the body with muscarine sulphate) would affect the tonus waves as did the muscarine in Fano and Sciolla's experiments. Incidentally, we tried the action of atropine and adrenalin upon the auricles which had been treated with pilocarpine.

METHOD

The method of procedure was the same as that employed in the first paper of this series (2). Thirty auricles were used. The drugs used were pilocarpine hydrochloride, atropine sulphate, and adrenalin. These were added to the Ringer's solution in which the muscles were contracting.

RESULTS

Pilocarpine hydrochloride. In only one animal were we able to get an increase in the tonus waves by the addition of pilocarpine hydrochloride and a decrease by the addition of atropine sulphate. In this auricle the addition of oxygen and the withdrawal of oxygen from the Ringer's solution produced a similar increase in the waves. We found that if the tonus waves were present pilocarpine appeared to exaggerate them somewhat, in a few cases, by slowing the rate of contraction and thus decreas-

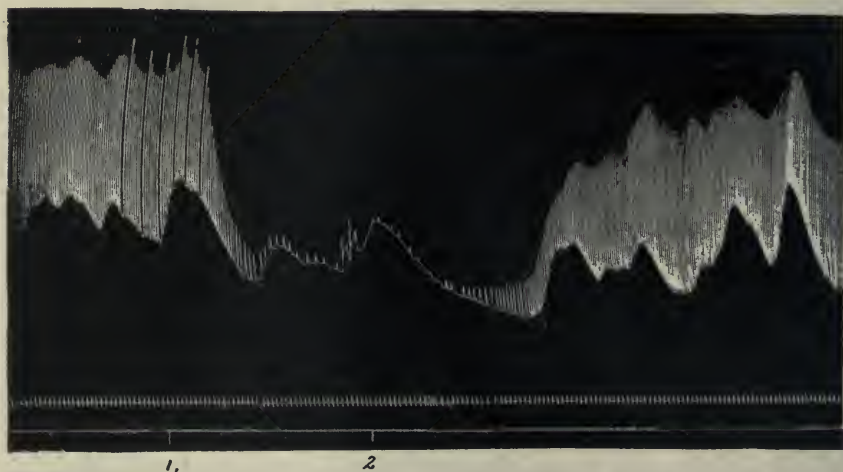


FIG. 1. BEAKER CONTAINED 80 CC. OF RINGER'S SOLUTION

In this and the following records the upper record is that of the contracting auricles of the species *Chrysemys cineria*; the middle record, that of the time interval in 5 seconds; and the lower, that of the time of the injection of the drug. The up stroke is the systolic contraction, the down stroke the diastolic contractions. All curves reduced $\frac{1}{3}$. 1, Pilocarpine hydrochloride, 1 mgm.; 2, atropine sulphate, 5 mgm.

ing the rebound of the lever. If the waves were not present, pilocarpine hydrochloride possessed no ability whatever to bring them about.

In figure 1 the tonus waves were present at the time of the addition of pilocarpine, 5 mgm. to 83 cc. of Ringer's solution (at 1). There resulted from the addition of the drug a marked decrease in the rate and height of contraction but no noticeable

increase in the tonus waves. There was, accompanying the decreased height of contraction, a loss of general tonus.

Pilocarpine hydrochloride, 1 and 2 mgm. respectively, was added to the 85 cc. of Ringer's solution in which the muscle was contracting (at 1 and 2) in figure 2. The first injection had no effect except, possibly, to increase the general tonus. The second injection brought about a decrease in the rate of contraction, the height of contraction, and the general tonus of the muscle.

Atropine sulphate. In figure 1 (at 2) 5 mgm. of atropine sulphate was added directly to the solution to which the pilocar-

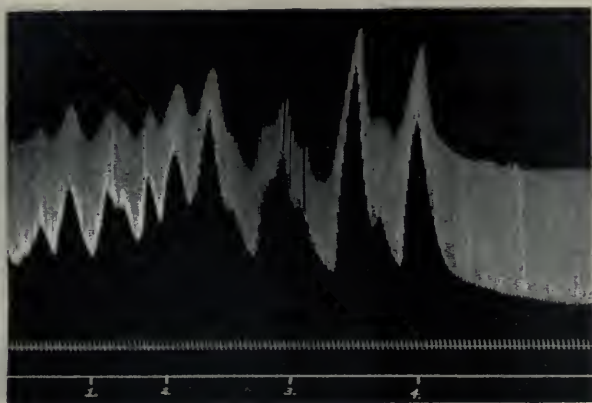


FIG. 2. BEAKER CONTAINED 85 CC. RINGER'S SOLUTION

1. Pilocarpine hydrochloride, 1 mgm.; 2, pilocarpine hydrochloride, 2 mgm.; 3, atropine sulphate, 15 mgm.; 4, adrenalin chloride 1 cc. of a 1:1000 solution.

pine had been previously added. The atropine did not cause a disappearance of the rhythmical oscillatory waves but counteracted the action of pilocarpine so that the contractions were more powerful and more rapid.

In figure 2 (at 3) atropine sulphate, 15 mgm., was added directly to the 85 cc. of Ringer's solution which still contained the pilocarpine hydrochloride. There resulted from this injection a marked increase in the depth of tonus waves and increased general tonus.

The sino-auricular muscle in figure 3 did not contract well in the beginning, probably because the vagi were still irritated by the excision of the heart. The contractions and waves having become fairly well established, atropine sulphate, 5 mgm. was added (at 1) to the 80 cc. of Ringer's solution. Here, as in the cases where pilocarpine was first injected, atropine did not bring about a cessation of the oscillations but increased the general tonus.

That adrenalin causes a cessation of the tonus waves in the atropinized muscle in exactly the same manner as in the normal

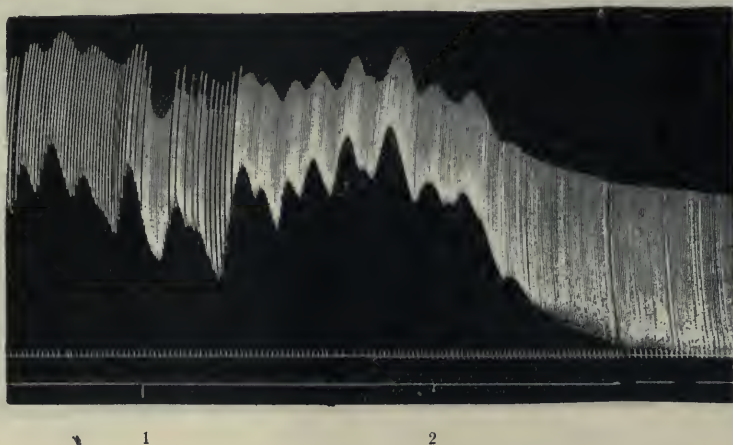


FIG. 3. BEAKER CONTAINED 80 CC. OF RINGER'S SOLUTION
1, Atropine sulphate, 5 mgm.; 2, epinephrin 1 cc. 1:1000 solution.

muscle can be seen in figure 2 (at 4) and in figure 3 (at 2). Adrenalin in solutions of 1:85,000 in the former case and 1:80,000 in the latter caused the oscillatory waves to disappear immediately.

DISCUSSION

It has been our experience that, if the tonus waves were not present, pilocarpine hydrochloride did not bring them about. This was the case in all the species of terrapin upon which we experimented. In eighteen animals of the species *Malacoclemmys lesuerii* and three of the *Chrysemys elegans* in which the

tonus waves were never very marked this drug had absolutely no bettering effect. In four hearts of the species *Chrysemys cineria* in which the tonus waves were not present, in three in which the tonus waves were not marked but present, and in six in which they were extremely pronounced, this drug had no bettering action whatever. As has been stated, we were able to demonstrate increased tonus due to pilocarpine in only one case, but the bubbling of oxygen through the solution and the withdrawal of oxygen had a similar effect. Atropine sulphate, contrary to Fano's and Sciolla's results did not cause a disappearance of the tonus waves except in the one case in which pilocarpine caused an increase.

SUMMARY

Pilocarpine hydrochloride has a marked effect upon the contractions of the heart, almost completely stopping them, but has no effect upon the tonus waves. It does not, like the muscarine of Fano's and Sciolla's experiments, increase them when present nor bring them about when absent.

Atropine sulphate increases the general tonus of the heart when injected into the solution in which the muscle is contracting, either in the normal solution or following the administration of pilocarpine. It has only a slight effect, if any, upon the tonus waves increasing them along with the general tonus.

Adrenalin chloride or epinephrin (crystalline adrenalin) causes a disappearance of the tonus waves in the atropinized heart as it does in the normal heart.

REFERENCES

- (1) FANO AND SCIOLLA: *Archives Italiennes de Biologie*, 1887-88, ix, p. 61.
- (2) GRUBER AND MARKEL: *This Journal*, 1918.

THE PRIMARY DEPRESSION AND SECONDARY RISE IN BLOOD PRESSURE CAUSED BY EPINEPHRINE

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Following the general blood pressure rise by epinephrine in many cases there is a distinct depression. This depression is much less than the rise and does not last as long. The cause of the depression has been investigated especially by Van Leersum (1), Hunt (2), Lohman (3), and especially by Weidlein (4). Weidlein has shown that with a carefully purified preparation of epinephrine no secondary depression occurs. According to him the depressant property of epinephrine is due to impurities present in the glands from which some extracts are prepared, and to the decomposition products. Hunt thinks that cholin is an important causative agent. Mostrom and McGuigan (5) consider that the agent which causes the depressant action may be formed in the animal either during or as a result of the rise, or as a decomposition product of the injected epinephrine. They also believe that the condition of the animal is of importance. It is known that glucose and lactic acid may be increased by the action of epinephrine and they have shown that acids may act as a contributing factor in causing the fall.

Cohen (6) has shown that acids have a well marked action on the central nervous system. Many investigators have shown that epinephrine has a direct central action and the present investigation shows that it also has an indirect central action due to pressure changes in the brain.

Preceding the depressant action, and following the rapid primary rise, there is a sharp primary fall followed, in most cases,

by a secondary rise (see fig. 1). It is to the primary fall and the secondary rise that the present investigation is directed. In the investigation we used adrenalin as the epinephrine preparation. The secondary rise falls gradually into the depressant state which was investigated by the authors quoted. One must infer from the literature that the two conditions in many cases have been confused. Yet we think that the action may be due to similar causes in each case. The results obtained by Cannon and Lyman (8) on the depressing effects of small doses of epinephrine, we think are exactly similar to the primary fall yet they quote the results of Weidlein which deals with the secondary fall as being similar with their own work. If one examines the tracings presented by Weidlein however, he will find both the primary and secondary fall in the same tracing and without question Weidlein had his attention focused on the secondary depression. The work of Lyman and Cannon with dilute solutions seems more comparable to our present investigation and may be identical with it. If one disregards the primary rise which we obtain with larger doses, the tracings of Cannon and Lyman might be superimposed in many cases and appear as the same tracing.

The following hypotheses for the cause of the primary fall and secondary rise have been considered:

1. That it is due to an action on the depressor mechanism which is not synchronous with the action on the constrictor mechanism. We think however, that this hypothesis is not adequate because the primary fall and the secondary rise occur after atropin, pilocarpin, and section of the vagi either before or after ligation of the carotids. That such a mechanism exists and is operative under special conditions was shown by Elliott (7). Cannon and Lyman (8) also give evidence that the depressor mechanism is not involved when the fall occurs after the administration of dilute solutions.

2. The primary fall and secondary rise might be due to a constriction and dilatation of the vessels of different organs at different times, the kidneys or the musculature, for example, not being acted upon synchronously. This explanation is not sufficient because ligation of the abdominal aorta below the kid-

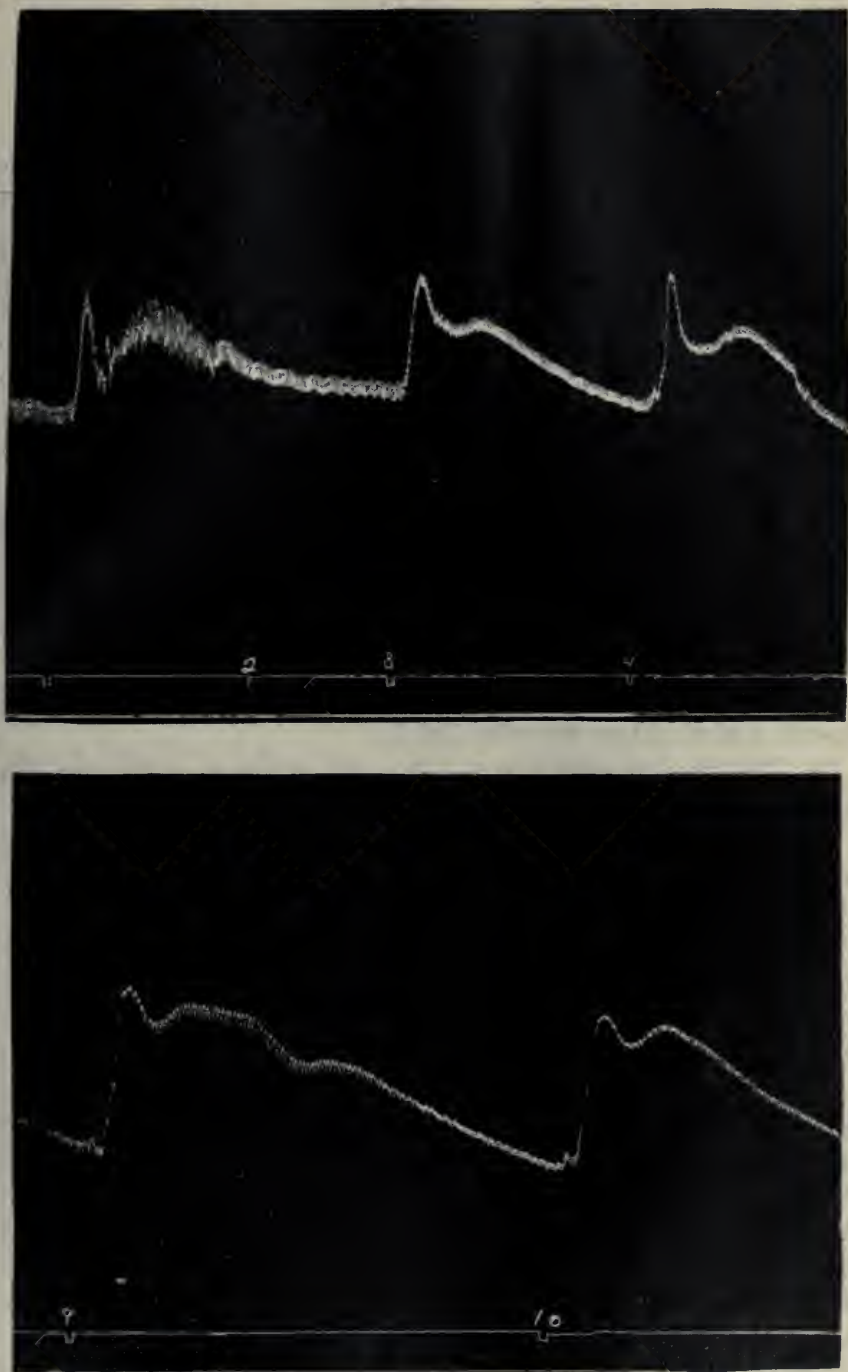


FIG. 1. ACTION OF ADRENALIN BEFORE AND AFTER LIGATION OF THE CAROTIDS AND SECTION OF THE VAGI

1, Injection of adrenalin, 0.5 cc., 1:10,000 dilution; 2, carotids clamped off; 3, adrenalin, 0.5 cc.; 4, adrenalin, 0.8 cc.; 9, adrenalin, 1.0 cc., 1:10,000, after section of the vagi; 10, adrenalin, 1.0 cc., 1:10,000, after section of the vagi.

neys, ligation of the kidneys, and ligation of the carotids does not materially change the blood pressure picture, and with all the peripheral circulatory mechanism intact, the phenomenon is absent after the ablation of the head or after pithing the brain. There may be however, some part played by this mechanism for Hoskins and Gunning (9) found that adrenalin in all effective doses causes a brief dilation followed by a contraction of the spleen. This however, was not manifest on the general blood pressure tracings probably because the methods of recording are not delicate enough to portray the rapidly changing conditions.

3. The primary fall may be due to a stimulation of the dilator mechanism which occurs later than the stimulation of the constrictors. The dilator mechanism soon fatigues and this, because of the continuous action of the constrictors, is the cause of the secondary rise. In favor of this hypothesis are:

a. The fact that the vaso-constrictors are in tonic activity and hence in a condition to react quickly.

b. The vaso-dilators are not in tonic activity and hence will respond more slowly.

c. A mechanism that is little used will fatigue readily. Hence repeated doses of epinephrine soon fail to elicit the primary fall. At this time larger doses may do so.

d. That the vaso-dilators are less responsive than the constrictors is shown by sectioning the mixed nerves, the dilators degenerating more slowly and retaining their irritability longer than the constrictors.

e. When the constrictors are paralyzed by apocodeine or by ergotoxine, epinephrine causes a fall only, because the dilators alone are stimulated and in such cases they should fatigue more readily. We have been unable to obtain any support for this part of the hypothesis and consequently we are forced to discard it. Also after large doses of nitrites the mechanism is paralyzed and we get no primary fall or secondary rise with epinephrine, at any stage of the paralysis.

4. The mechanism involved in the primary fall and the secondary rise might be due to the action of the injected epinephrine on the adrenal glands. The primary rise being due directly to

the action of the injected drug on the peripheral vessels, as the constrictor effect of this begins to wear off, the increased secretion of epinephrine by the glands due to the injected epinephrine, begins to act and consequently we get a secondary rise. This theory is somewhat supported by the fact that removal of the glands prevents to a considerable degree, the development of the secondary rise. Increasing the dose of the injected epinephrine however, will, in most cases, bring out the phenomenon. Apparently then the only influence of removing the adrenal glands is a shock effect and a lessening of the sensitivity of the reactive mechanism. Nicotin after removal of the glands has much less influence in raising the blood pressure, but still prevents the secondary rise.

5. Epinephrine has both a peripheral and a central action. Many investigators (7) have given proof of central action. The action on each point is not quite synchronous and when the drug begins to lose its peripheral action and the primary fall commences, the vaso-constrictor center is attacked and we get the secondary rise. In favor of this theory are the facts:

a. That after morphine or other central paralysing drug we get no secondary rise, or it is greatly modified.

b. After the removal of the head or high section of the cord we get no secondary rise.

c. In depressed conditions of the cord from any cause it may be absent.

d. When the centre is so depressed that the usual small dose does not give the secondary rise, a larger dose may bring out the action.

e. In depressed states strychnine may aid in bringing out the reaction.

f. When the depressor nerve is stimulated during the injection of dilute adrenalin, there is a greater fall of pressure than is caused by the depressor stimulation alone (Cannon and Lyman, loc. cit.).

g. Hartman and Kilborn (10) have shown that in young kittens, adrenalin exerts only a depressor effect, due perhaps to failure in the development of the central nervous mechanism.

We have found also, that so far as the primary fall and secondary rise is considered young animals are unreliable and variable, and in most cases show no primary depression.

After a consideration of all the hypotheses given, we conclude that the primary rise is due entirely to peripheral action. Oncometer tracings of the peripheral organs show uniformly a constrictor effect which is synchronous with the rise in blood pres-



FIG. 2. THE EFFECT OF ADRENALIN AFTER NICOTIN, IN LARGE DOSES; SMALL DOSES MAY ACCENTUATE THE SECONDARY RISE.

The curve is the same as that obtained after removal of brain.

sure. The primary fall if uninfluenced by central action would continue to fall and the tracing would show simply a rise and a fall without variation. However, blood pressure changes within the brain or connected with the circulation of the brain, change the primary fall so that in many cases a secondary rise occurs. This secondary rise is we think due specifically to blood pressure changes in the brain. The central action occurs later and is prevented by pithing or by removal of the head (fig. 2). Section

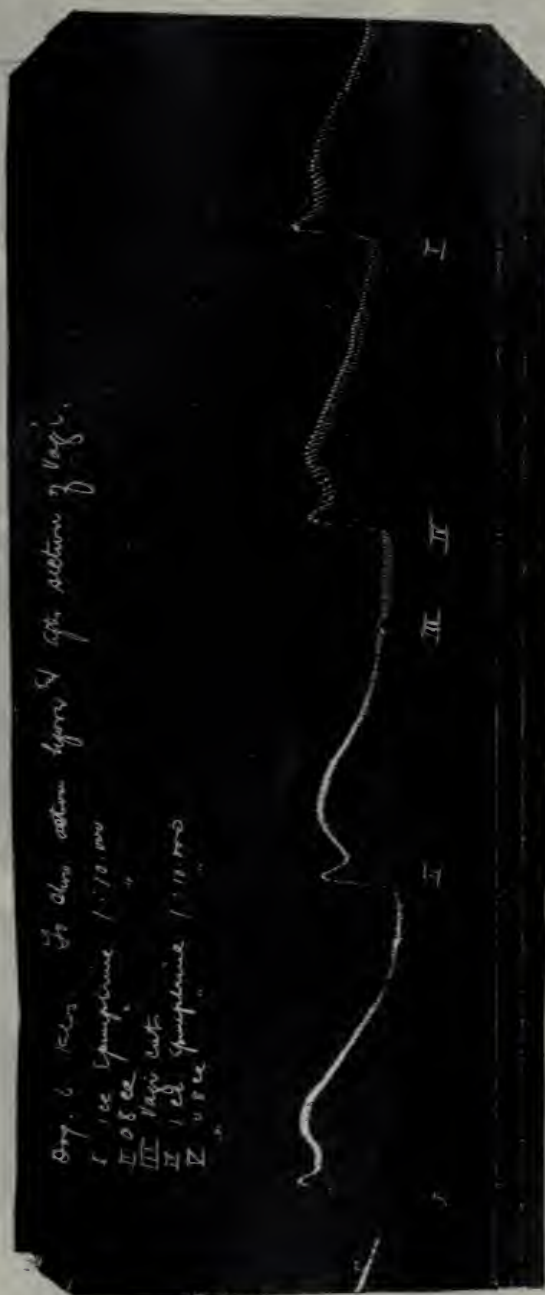


FIG. 3. ACTION OF EPINEPHRINE BEFORE AND AFTER SECTION OF VAGI

1, 1 cc. epinephrine, 1:10,000; 2, 0.8 cc. epinephrine, 1:10,000; 3, vagi cut; 4, 1 cc. epinephrine, 1:10,000; 5, 0.8 cc. epinephrine, 1:10,000; dog, 6 kilos.

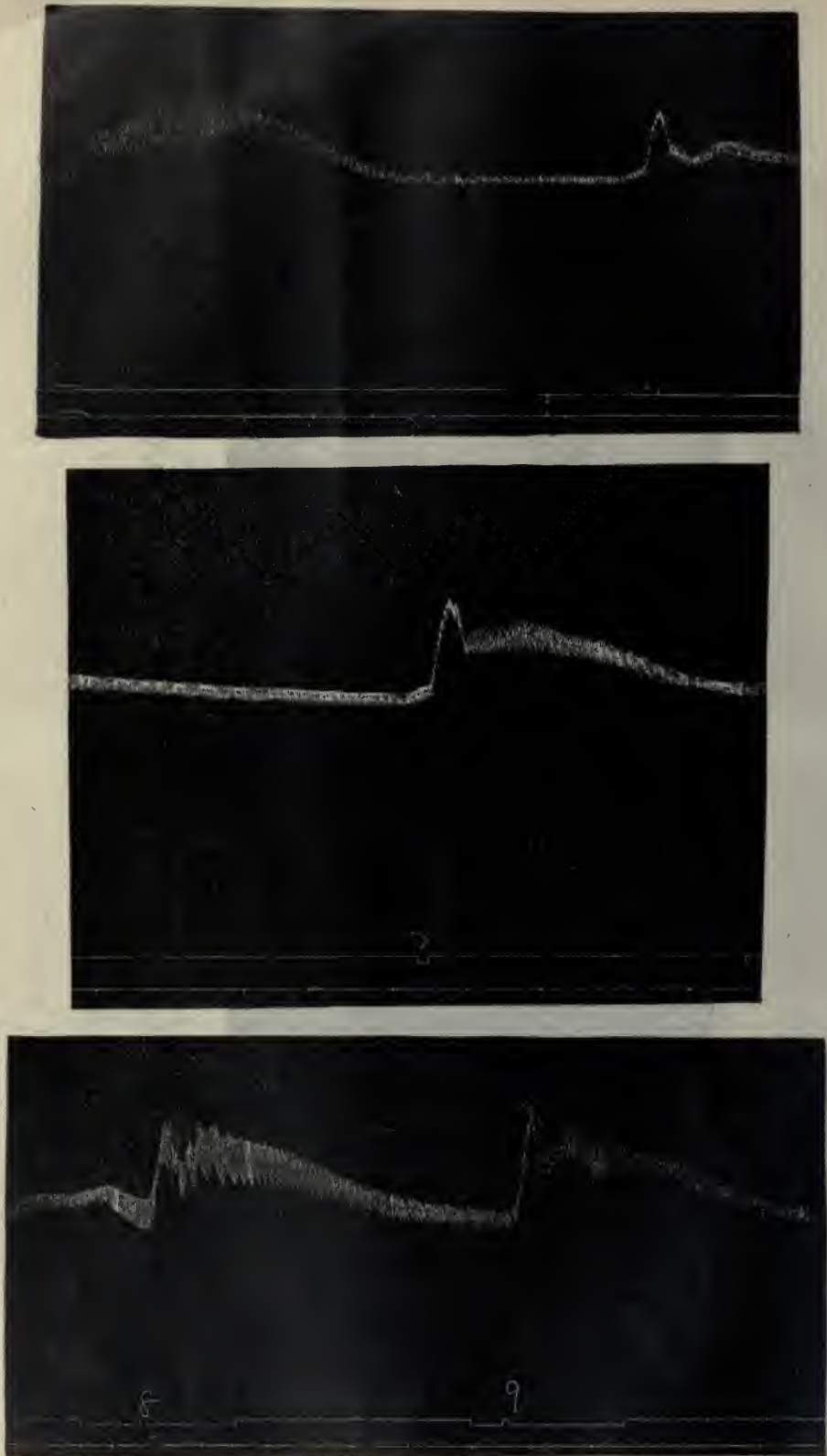


FIG. 4. THE EFFECT OF EXTRA-DURAL PRESSURE

5, Normal tracing, adrenalin 1: 10,000; 6, tracing during negative pressure; 7, normal tracing, adrenalin 1: 10,000; 8, tracing during positive pressure, 9, tracing during positive pressure.

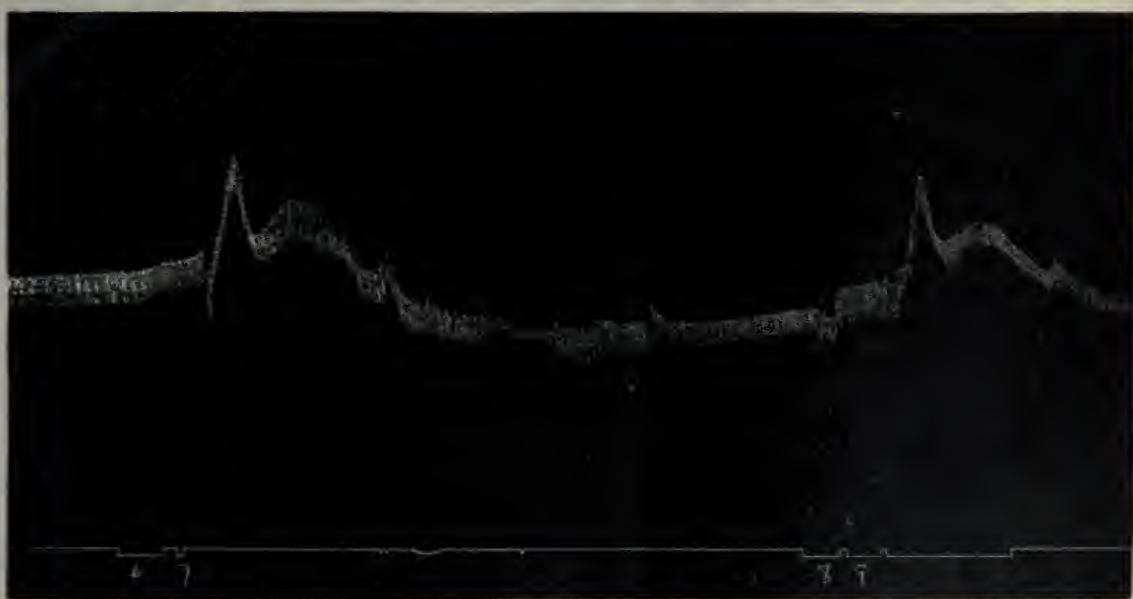
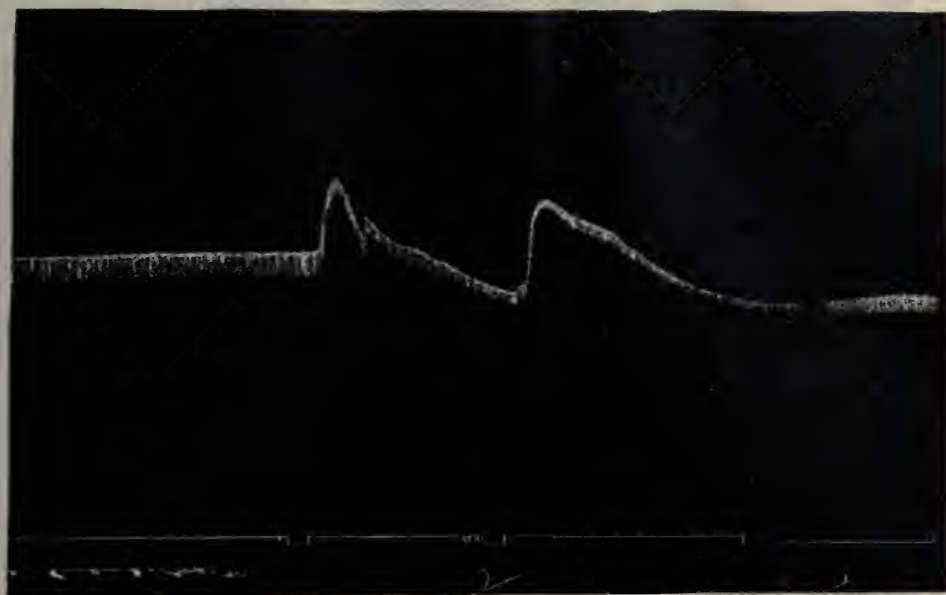


FIG. 5. THE EFFECT OF INCREASING THE PRESSURE OF CEREBROSPINAL FLUID IN THE FOURTH VENTRICLE
 1, Normal tracing of adrenalin 1:10,000; 2, normal tracing of adrenalin 1:10,000; 6 and 8, the effect of increasing pressure in the fourth ventricle; 7 and 9, injection of adrenalin 1:10,000 during increased pressure in the fourth ventricle.

of the vagi or atropin does not prevent it (fig. 3). In many cases section of the vagi accentuates the secondary rise. Nicotine, given until the ganglia are paralysed, prevents the recurrence of the phenomenon; it seems therefore, that the mechanism is due to a central action which is conveyed through the sympathetic ganglia. Smaller or stimulating doses of nicotine may accentuate the primary fall and secondary rise. By changing the intracranial pressure with a water manometer through a trephine hole in the skull, a modification of the blood pressure to give a typical secondary rise can be obtained (see fig. 4). A greater increase in intracranial pressure however may again prevent the secondary rise. Changes within the cerebro-spinal fluid also modify the blood pressure tracing of epinephrine (fig. 5). The details of this pressure effect and the actual pressure required have not yet been completely investigated. It is apparently not a direct action of epinephrine on the brain because when injected into the fourth ventricle there is no effect, and when injected into the carotid artery, the changes occur later than if the injection be made into the vein.

SUMMARY

In most dogs, especially mature animals in good health, the intravenous administration of adequate doses of epinephrine (0.5 to 1 cc. of 1:10,000), after a quick rise in the blood pressure is followed by a rapid fall and a secondary rise. Various hypotheses were investigated. The cause of the secondary rise is apparently due to a central action of the epinephrine acting through the sympathetic ganglions. The basis for this belief is that the removal of the head or pithing of the brain prevents the occurrence of the phenomenon. Also paralysis of the ganglia with nicotin prevents it. It occurs after the sectioning of the vagi and the administration of atropin or pilocarpin. The vagus apparently is not involved in the mechanism. Artificial intracranial pressure during the administration of the epinephrine will cause similar changes in the blood pressure.

REFERENCES

- (1) VAN LEERSUM: Pflüger's Archiv, 1911, cxlii, 377.
- (2) HUNT: Amer. Jour. Physiol., iii, 17 and v, 6, Proceedings.
- (3) LOHMAN: Pflüger's Archiv, 1907, cxviii, 215.
- (4) WEIDLEIN: Jour. Ind. and Eng. Chem., 1912, iv, 640.
- (5) MOSTROM AND MCGUIGAN: Jour. Pharm. and Exp. Therap., 1913, iv, 277.
- (6) COHEN: Jour. Pharm. and Exp. Therap., 1918, xi, 221.
- (7) ELLIOTT: Jour. Physiol., 1905, xxxii, 428.
DIXON: Jour. Physiol., 1903, xxx, 115.
PRUSZYNSKI: Archiv. f. (Anat. u.) Physiol., 1903, 522.
WEBER: Schmidt's Jahresbuch ges. Med., 1904, cclxxxiv, 175.
MAGNUS: Archiv Exp. Path. u. Pharm., 1903, 1, 86.
- (8) CANNON AND LYMAN: Amer. Jour. Physiol., 1913, xxxi, 476.
- (9) HOSKINS AND GUNNING: Amer. Jour. Physiol., 1917, xliii, 299.
- (10) HARTMAN AND KILBORN: Amer. Jour. Physiol., 1918, lv, 117.
HARTMAN: Endocrinology, 1918, ii, 1.

THE EFFECTS OF VARIOUS AGENTS ON SUPERFICIAL HEMORRHAGE AND THE EFFICIENCY OF LOCAL HEMOSTATICS

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There is a variety of agents described as local hemostatics in textbooks of pharmacology and therapeutics, whose activity may be questioned owing to a dearth of scientific evidence. The reputations enjoyed by many of these have been acquired chiefly empirically. The same may be said of a number of commercial products recommended for use in the treatment of hemorrhage (local and systemic) due to a variety of causes, and by practically all known channels of administration. The claims made for the hemostatic efficiency of stypticin and styptol (both salts of cotarnine) have not been substantiated by several investigators who approached the problem from different points of view and also by the method of one of their warmest advocates. It seems scarcely warranted, to rest the subject on opinion, uncontrolled empirical observations and numerous vagaries which have extolled many of these remedies that fail when subjected to crucial tests. The lack of a suitable method is perhaps responsible for the almost total lack of scientific investigations in this field. In view of these deficiencies, it seemed worth while to investigate this class of agents by a method which could provide suitable controls and give a quantitative estimate of their comparative value.

METHOD

In a previous publication (1) it was pointed out that inspection alone (as practiced by K. Abel) cannot give an accurate idea of the nature of the effects or the variability of hemostatics when

applied directly to bleeding wounds. However, the one feature of the original method of K. Abel, namely, the use of the incised foot-pad as a test object is convenient and admirably suited for use in the procedure to be described. Briefly, this new method consists of continuous irrigation of the denervated and incised foot-pad of the dog with a non-coagulant solution, with and without the hemostatic, collecting the wash fluid for a definite period at intervals, and estimating a constituent of the blood in the wash-fluid.

In detail the method may be described as follows; medium sized dogs of about 6 to 8 kilograms previously anesthetized with morphin (0.02 gram per kilogram) and Gréhan's anesthetic¹ are best suited for the purpose. With this kind of anesthesia the animal lies perfectly quiet throughout the experiment, a condition that is nearly ideal and indispensable in order to avoid the disturbing influences of various systemic and local changes brought about by struggling and reflexes. Blood pressure is registered in the usual way from the carotid artery. It is essential to observe the changes in blood pressure because this is probably the most important external factor to influence the character of the bleeding, the changes taking place in the same direction. Next the saphenous and sciatic nerves are severed in order to exclude central vasomotor influences on the vessels in the pad. This also tends to maintain the bleeding more constant.

The superficial skin of the central pad is now incised with a sharp scalpel so that the papillary layer is exposed leaving behind a smooth surface which is promptly covered with oozing blood. The fat pad proper should not be uncovered. The entire hairless area is incised so as to obtain a fairly good sized wound, ranging from about 2 to 4 sq. cm. However, the exact area is immaterial. The wound is now continuously irrigated with 1 per cent citrate from a vessel (perfusion-bottle) situated about 50 cm. above a pan of water maintained at a constant temperature (50°C.) by means of an electric stove. The citrate is caused to circulate

¹ The Gréhan mixture used consisted of 25 cc. of chloroform in one liter of 50 per cent alcohol, and the dosage was 9 to 10 cc. per kilogram by stomach one-half hour after the administration of morphin.

through a glass tube (in the form of a U) immersed in the water-bath and then by means of rubber tubing is joined to a bent glass tube which delivers the warmed citrate (at 37.5° to $38^{\circ}\text{C}.$) about 10 cm. above the wound at the rate of about 10 cc. per minute (about 40 drops in 15 seconds). The rate of flow is conveniently regulated by means of a screw stop-cock. A small pan is placed underneath the pad to collect the wash fluid between collections for analysis. A second reservoir with a capacity of about 200 cc. is placed and fitted in the same way as the citrate reservoir, with the delivery tube closely situated to the citrate delivery for convenience in making changes from the plain to the treated citrate contained in the smaller reservoir.

Collections of the wash-fluid are now made for definite periods of one-half minute each and at intervals of 1 to 5 minutes depending on the nature of the experiment. A control is first secured by making four or five collections at intervals of 5 minutes or until the hemorrhage is constant which can be roughly judged by the appearance of the wash-fluid. When the bleeding appears to be constant, the citrate containing the agent to be tested is quickly applied and irrigation of the wound continued for any desired length of time. In this work irrigations varied from 15 seconds to 5 minutes depending on the nature of the experiment and collections were generally made at 5-minute intervals and as long as the effects lasted or until bleeding returned to approximately its previous level.

When the effects of a drug have disappeared, and the bleeding has about returned to its previous level, collections at 5 minute intervals are continued for about 15 to 20 minutes, and these serve as controls for the next drug to be applied. In this way repeated controls are obtained and the same wound may be used continuously for different drugs, and despite changes in the blood pressure. I have been able to observe such a wound for 8 hours and study the effects of twelve different agents, obtaining numerous controls in between and the total number of collections of wash-fluid amounted to 75. Towards the end of the experiment the blood pressure falls, but with sufficient controls it is possible to continue observations until the pressure falls to

15 to 20 mm. The real value of the systemic blood pressure measurements is during the act of irrigation and action of the hemostatic.

What has been said refers to agents which do not coagulate or destroy tissue, such, for instance, as epinephrin. With the various astringents, such as tannin and the metallic salts, it is practically impossible to use the wound after a single application of one of these, but this will vary somewhat with the strength of the styptic. In case the treated wound ceases to bleed the other foot-pad may be utilized. The front feet are not so convenient. If it is, therefore, desirable to demonstrate the effects of a series of agents on local hemorrhage it is well to begin with those which have no coagulant or destructive action on tissues and end up with an astringent. This, in general, has been the plan adopted in the experiments here reported.

Quantitative index of the hemorrhage. This is obtained by estimating some constituent of the blood whose content remains fairly constant. The estimation of hemoglobin would be the most desirable. This was made use of by Lisin (2) and Frey (3) in their studies on systemic hemostatics. However, the direct treatment of the wounds with various astringents which precipitate the blood, and colored agents, precludes the colorimetric estimation of hemoglobin. For these reasons also the counting of erythrocytes, the colorimetric estimation of iron and the determination of total solids are precluded. This leaves urea-nitrogen, which is not affected by any of these agents, as the most desirable. For this, the urease-aeration-colorimetric method is most convenient and practical, rendering it possible with suitable apparatus to make a great many estimations (60 to 70) in a single day.

It is necessary to observe the following precautions in the application of the method. The rate of citrate flow should not exceed approximately 10 cc. per minute, for, if this is exceeded, the total volume of the wash-fluid in the long test tubes used for aeration is too large and impairs the accuracy, the removal of the ammonia being incomplete. If the reaction of the wash-fluid has been altered to acid after treatment of the wound with such

agents as tannin, ferric chloride and other metallic astringents, also pituitary extracts, which are apt to be quite acid, the fluid must be neutralized before the addition of the urease. Strongly alkaline fluids must also be neutralized. In the beginning of the experiment the quantity of urea in a single collection of wash-fluid is equivalent to that of about 1 to 2 cc. of the animal's blood. The quantity of blood present will vary with the extent of the wound area and vessels involved, and in an experience with about sixty animals, this has required no more urease than is ordinarily used in the analysis of the average sample of human blood used in renal function tests. The wash-fluid is transferred from the beaker directly to the long test tubes, washed with about 5 cc. of water and the glycerin urease extract² (0.5 cc.) is added using two drops of caprylic alcohol to avoid foaming, the entire technique being carried out in the usual way.

Comparison of urea-nitrogen with hemoglobin estimations. Several comparisons of the urea-nitrogen with the hemoglobin content of the same wash-fluid from bleeding wounds were made and the results that are presented in the form of curves in figure 1, which illustrates the effects of a number of agents, prove conclusively that either constituent can serve as a quantitative index of the hemorrhage. The hemoglobin was estimated colorimetrically by Palmer's (4) method, assigning the value of 100 per cent to the first collection of wash-fluid. The remaining results, therefore, represent relative percentages. Because of its general applicability with the various agents used and for purposes of uniformity and comparisons the urea-nitrogen of the blood was selected as the method of choice.

It must be obvious that the results obtained by this method of studying local hemorrhage can not be transferred directly to normal or untreated wounds, or in various clinical conditions. It may be doubted if the class of hemostatic products derived from tissue and organ extracts, such as kephalin, and whose actions involve factors concerned in the natural clotting of blood, could be estimated from this standpoint under the conditions.

² This was suggested by Dr. C. H. Fiske of the Biochemical Laboratory.

However, these products have been studied almost exclusively as to their effects on coagulation time and frequently in vitro alone. Therefore, other actions (particularly with higher concentrations) which they may possess, and be concerned in hemostasis will be indicated by the method used in this study.

On the other hand, the results obtained with various vascular agents and nearly all the astringents tested produced effects which

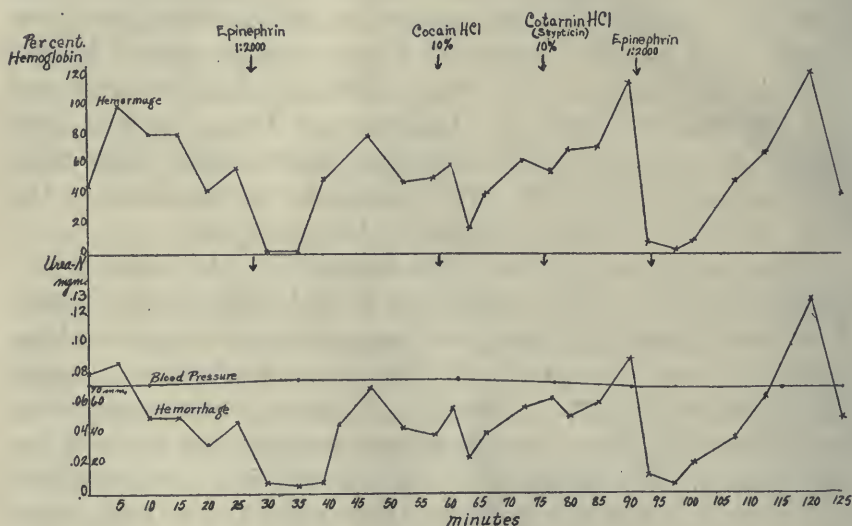


FIG. 1. EXPERIMENT 34. COMPARISON OF THE HEMOGLOBIN AND UREA-NITROGEN CONTENT OF BLOOD AND THE EFFECTS OF DIFFERENT LOCAL AGENTS, DURING THE COURSE OF HEMORRHAGE FROM THE DOG'S FOOT-PAD

Irrigations with 1 cc. for 15 seconds were made with each solution.

agree with their ordinary actions. Furthermore, if a drug has a demonstrable effect under unfavorable conditions it is likely that its qualitative effects would not be seriously modified under favorable conditions. With some of the agents whose ordinary actions might conceivably be modified, slight modifications of the original method were made and these will be referred to in the text. The method is useful in judging of the comparative value of these agents.

EXPERIMENTAL RESULTS

At least three applications to the same or different wounds were made with the majority of the 62 different agents tested, and in many cases many more applications were made. The results (both as to bleeding and blood pressure) have been tabulated and curves of all the experiments constructed and from these the effects as to quality and comparative efficiency have been judged and estimated. All results which were seen to be influenced by changes in blood pressure have been eliminated. The effects of all the agents studied are summarized in table 1.

Table 2 illustrates the general classification of the agents tested as to their effects on local hemorrhage. Those showing uniform changes of less than 10 per cent have been classed in the "Unchanged" column.

For convenience and brevity the various agents may be discussed in groups, which, however, do not always indicate the mechanisms of action involved. Certain of the agents are of special interest because of the hemostatic actions displayed, others because of their notoriety, and still others because of items with no direct bearing on hemostasis.

Epinephrin and related agents

Epinephrin. The results from 27 applications showed that with concentrations ranging from 1:1,000,000 to 1:1000 epinephrin promptly and effectively lessens or arrests (by 28 to 100 per cent) the flow of blood, although temporarily. The maximal effect appeared within an average of $2\frac{3}{4}$ minutes, and the duration of the action depends on the period of irrigation and concentration of epinephrin used, being longer with higher and shorter with low concentrations and roughly proportional to the period of irrigation (see fig. 2). The action begins almost immediately with the application of the drug, the wound tissue appearing smooth, white and glistening when the effect is fully developed and providing large vessels are not involved.

TABLE 1

Effects of various agents (locally applied) on local hemorrhage from the dog's foot-pad

A. Lessened hemorrhage

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECT	REMARKS
Epinephrin								
29(2)	1: 1000	$\frac{1}{4}$	-	30	$2\frac{1}{2}$	- 67	Slow +	
30	1: 1000	$\frac{1}{4}$	-	11	5	-100	+	
30	1: 1000	$\frac{1}{4}$	-	17	5	- 71	Normal	
31	1: 1000	$\frac{1}{4}$	-	27	7	- 75	+	
31	1: 1000	$\frac{1}{4}$	-	20	2	- 28	+	
31	1: 1000	$\frac{1}{4}$	-	22	$2\frac{1}{2}$	- 40	+	
32	1: 1000	$\frac{1}{4}$	-	19	3	- 86	+	
32	1: 1000	$\frac{1}{4}$	-	15	4	- 86	+	
32	1: 1000	$\frac{1}{4}$	-	17	$2\frac{1}{2}$	- 90	Slight+	
33	1: 1000	1	-	18	$2\frac{1}{2}$	- 50	Normal	
34	1: 2000	$\frac{1}{4}$	-	16	2	- 80	+	
34	1: 2000	$\frac{1}{4}$	-	22	1	- 83	+	
35	1: 1000	$\frac{1}{4}$	-	23	3	- 95	+	
35	1: 1000	$\frac{1}{4}$	-		6	- 92		
36	1: 1,000,000	1	-	3	$2\frac{1}{2}$	- 36	+	
36	1: 100,000	1	-	7	$2\frac{1}{2}$	- 54	+	
36	1: 10,000	1	-	$14\frac{1}{2}$	3	- 76	+	
36	1: 1000	1	-	$52\frac{1}{2}$	$2\frac{1}{2}$	-100		
37	1: 50,000,000	$1\frac{1}{2}$	+	3	3	+ 23	Normal	
38	1: 50,000,000	1	+	4	3	+ 13	Normal	
38	1: 10,000,000	1	+	11	$2\frac{1}{2}$	+ 4	Normal	
38	1: 1,000,000	1	-	2	1	- 50		
39	1: 50,000,000	1	+	$5\frac{1}{2}$	$2\frac{1}{2}$	+ 18	Slight-	
39	1: 10,000,000	1	+	17	$2\frac{1}{2}$	+ 58	Normal	
39	1: 1,000,000	1	-	19	4	- 22	+	
39	1: 100,000	1	-	16	10	- 80	+	
39	1: 10,000	1	-	47	4	- 90		
39	1: 1000	1	-	70+	10	-100	Normal	
41	1: 1000	$\frac{1}{2}$	-	30+	1	- 90		
41	1: 1000	1	-		1	- 89		
41	1: 1000	1	-	8	1	- 75	+	

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECTS	REMARKS
<i>Epinephrin—Continued</i>								
29 (1)	1: 1000	$\frac{1}{4}$ min-utes	—	33 min-utes	8 minutes	- 69 per cent	+	
42	1: 1000	$\frac{1}{2}$	—	15+	$\frac{1}{2}$	-100		
43	1: 1000	2	—	7	$\frac{1}{2}$	- 64	+	
44	1: 1000	$\frac{1}{2}$	—	40+	11	- 73		
55	1: 1000	5	—	80+	$\frac{1}{2}$	- 82		
55	1: 1000	5	—	100+	$\frac{1}{2}$	- 41		After 5 per cent tan- nin
<i>Barium chloride</i>								
32	1.0 per cent	$\frac{1}{4}$	—	1	1	- 10	Slight+	
46	10.0 per cent	5	—	60	10	-100	Slight+	
61	10.0 per cent	5	—	45	20	- 66		
<i>Acetic acid</i>								
42	0.1 per cent	$\frac{1}{2}$	—	8	8	- 60		
42	0.1 per cent	5	—	20+	1	-100		
<i>Hydrochloric acid</i>								
53	1.0 per cent	5	—	15+	15	- 84		
57	1.0 per cent	5	+	11+	10	- 53		
<i>Sodium bicarbonate</i>								
57	1.0 per cent	5	—	6	1	- 33	+	
58	2.0 per cent	5	—	15	10	- 40	Normal	
61	0.5 per cent	5	—	15+	15	- 31		In citrate 0.5 per cent
<i>Cocain hydrochloride</i>								
31	10.0 per cent	$\frac{1}{4}$	+	7	2	+260	Normal	
	1.0 per cent	$\frac{1}{4}$	+	$3\frac{1}{2}$	1	+ 33	Normal	
32	1.0 per cent	$\frac{1}{2}$	+	13	6	+ 50		

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECTS	REMARKS
Cocain hydrochloride—Continued								
34	10.0 per cent	$\frac{1}{2}$	First+	min- utes	minutes	per cent		
			Later--					
35	1.0 per cent	1	—	9	4	+ 38		
	5.0 per cent	1	—	14+	6	- 45		
39	1.0 per cent	1	—	10	6	- 61		+ Slight+
41	1.0 per cent	5	—	17	7	- 43		Slight+
	1.0 per cent	5	—	22	1	- 100		+
	1.0 per cent	5	—	15+	1	- 50		
						- 50		
Tincture of digitalis (alcohol-free)								
43	10.0 per cent	1	—	30	30	- 67	Normal	
	10.0 per cent	5	—	10	5	- 86	Normal	
44	10.0 per cent	1	—	3	2	- 60	Normal	
	10.0 per cent	5	—	12	12	- 50		
45	10.0 per cent	5	—	25	25	- 61	Normal	
Tincture of strophanthus (alcohol-free)								
43	10.0 per cent	1	—	10	9	- 30	Normal	
	10.0 per cent	5	—	7	7	- 29	Normal	
44	10.0 per cent	1	—	12	5	- 19	Normal	
	10.0 per cent	5	Un- changed					
45	10.0 per cent	5		12	8	- 61	Normal	
Caffein								
47	1 per cent	5	—	20	10	- 82	Normal	
48	1 per cent	5	—	3	1	- 33	Normal	
58	1 per cent	5	—	17	6	- 73	Normal	
Tyramin								
51	0.1 per cent	5	—	5	1	- 66	Normal	
	0.2 per cent	5	—	11	6	- 58	Normal	
48	0.1 per cent	$1\frac{1}{2}$	—	12	7	- 70		+
52	0.2 per cent	5	—	10	1	- 67	Normal	
55	0.5 per cent	5	—	15	6	- 55	Normal	

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECTS	REMARKS
Quinine hydrochloride								
49	0.1 per cent	5	—	7	5	— 43	Normal	
Quinine-urea hydrochloride								
52	1.0 per cent	5	—	15	5	— 36	Normal	
53	1.0 per cent	5	—	15+	10	— 38	Normal	
61	1.0 per cent	5	—	20	15	— 70		
Effects of cold								
35	1.0 per cent	1	—	5+	2	— 50	Normal	24°C.
55	1.0 per cent	5	—		$\frac{1}{2}$	— 50	Normal	21°C.
Zinc sulphate								
56	5.0 per cent	5	—	10+	6	— 53		
63	5.0 per cent	5	—	7	1	— 14	Normal	
Morphine sulphate								
51	1.0 per cent	5	—	15	1	— 56	Normal	
52	0.1 per cent	5	—	15	1	— 55	Normal	
61	0.1 per cent	5	—	11	6	— 6	Normal	
Ferric chloride								
50	1.0 per cent	1	—	15	5	— 22		
	1.0 per cent	5	—	40	15	— 100	Normal	
62	1.0 per cent	5	—	6	1	— 33	Normal	
Pituitary extract								
31	Lilly's	$\frac{1}{4}$	—	3	1	— 70	Normal	
33	Lilly's	$\frac{1}{2}$	—	8	5	— 65	Normal	
42	Lilly's	1	—	25	12	— 100	Normal	
43	Mulford's	$\frac{1}{2}$	—	13+	13	— 73		
		1	—		8	— 24		

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECTS	REMARKS
Pituitary extract—Continued								
		min-utes		min-utes	minutes	per cent		
44	Mulford's	1	{ first+ later-	3½	2	+ 50	Normal	
46	Armour's	1		6	3½	- 72		
49	Armour's	1	+	17	12	- 77	Normal	
51	Mulford's	5	+	17	14	+ 34	Normal	
				8	½	+ 61	Normal	
Calcium chloride								
55	2.0 per cent	5	-	11	6	- 12	Normal	In N.S.
	2.0 per cent	5	-	11	5	- 9	Normal	
49	2.0 per cent	5	-	15+	6	- 57		In H ₂ O
Oil of turpentine								
51	Whole	5	-	5	½	- 33	Normal	
Tannin								
54	5.0 per cent	5	-	15+	15	- 100	Normal	Permanent arrest
41	1.0 per cent	1	+	12	7	+ 66		
62	1.0 per cent	5	-	15	8	- 67	Normal	
62	5.0 per cent	5	-	30+	1	- 95		
Glycerite of tannic acid (20 per cent)								
30	U. S. P.	¼	-	12	10	- 60	Normal	Permanent hemostasis
41	U. S. P.	1	+	13	½	+ 270		
20	U. S. P.	5	+	8	2	+ 110	Normal	
61	U. S. P.	5	-	100+	25	- 100		
Cane-sugar								
50	10.0 per cent	5	-	11	2	- 60		
57	10.0 per cent	5	-	12	2	- 40		

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (— = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (—) OR INCREASE (+)	AFTER EFFECT	REMARKS
Peptone								
		minutes		minutes	minutes	per cent		
56	10.0 per cent	5	—	21+	16	— 54	Normal	
58	10.0 per cent	5	—	17+	6	— 52	Normal	
Silver nitrate								
63	1.0 per cent	5	—	15	3	— 75	Normal	
Sodium chloride								
49	0.9 per cent	5	—	10	1	— 90	Normal	Without citrate
50	0.9 per cent	5	—	5	1	— 50		
53	0.9 per cent	5	—	15	1	— 94	Normal	
57	10.0 per cent	5	—	11	Immediate	— 33		
57	10.0 per cent	5	—	20	Immediate	— 45	Normal	In citrate
58	10.0 per cent	5	—	16	6	— 25		
58	10.0 per cent	5	—	16+	16	— 50	Low	In citrate
58	0.9 per cent	5	—	11+	1	— 35		In citrate
58	0.9 per cent	5	—	15	10	— 38		In citrate
B. Increased hemorrhage								
Beef serum								
58	Whole	5	+	6	2	+ 45	Normal	Without citrate
	Whole	5	+	8	1	+ 66	Normal	With citrate
Horse serum								
59	Whole	5	—	20	11	— 44	Normal	Without citrate
60	Whole	5	Unchanged in 16				+	With citrate
	Whole	5	+	20	1	+125		Old serum without citrate

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECT	REMARKS
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Horse serum—Continued

		<i>minutes</i>		<i>minutes</i>	<i>minutes</i>	<i>per cent</i>		
60	Whole	5	+	16+	1	+ 80	+	Old serum with citrate
60	Whole	5	-	21+	1	- 60	-	Old serum without citrate
60	Whole	5	+	21	1	+ 68	+	Without citrate, containing 0.2 per cent trikresol
60	Whole	5	+	16	1	+210	Normal	With citrate containing 0.2 per cent trikresol
61	Whole	5	+	11	1	+ 70	Normal	With citrate containing 0.2 per cent trikresol

Hydrastinine hydrochloride

42	0.1 per cent	1	+	22	17	+ 44	Normal
53	0.1 per cent	5	+	11	$\frac{1}{2}$	+ 50	

Dichloramin-T

54	Saturated	5	+	16	$\frac{1}{2}$	+253	Normal
	Saturated	5	+	6	1	+ 30	Normal
61	Saturated	5	+	6	1	+ 67	Normal

TABLE 1—*Continued*

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECT	REMARKS
Chlorazene								
		min-utes		min-utes	minutes	per cent		
52	1.0 per cent	5	-	11	11	- 70		
54	1.0 per cent	5	+	3	$\frac{1}{2}$	+250	Normal	
58	1.0 per cent	5	+	15	$\frac{1}{2}$	+233	Normal	
58	2.0 per cent	5	+	22	$\frac{1}{2}$	+200	Normal	
Dakin's hypochlorite solution								
63	Whole	5	+	12	1	+540	Normal	Without citrate
63	Whole	5	+	15	1	+ 80	Normal	With citrate
Chloral hydrate								
47	5.0 per cent	5	+	6	4	+ 25	Normal	
48	5.0 per cent	5	+	8	1	+200	Normal	
57	5.0 per cent	5	+	11	$\frac{1}{2}$	+ 71	Normal	
	5.0 per cent	5	+	5	1	+ 25	Normal	
Sodium salicylate								
51	1.0 per cent	5	+	8	3	+ 15	Normal	
Cotarnine hydrochloride (stypticin)								
29	10.0 per cent	$\frac{1}{4}$	+	7+	$2\frac{1}{2}$	+218	Normal	
30	10.0 per cent	$\frac{1}{4}$	+	15	10	+163	Normal	
31	10.0 per cent	$\frac{1}{4}$	+	15	5	+ 52	Normal	
32	1.0 per cent	$\frac{1}{4}$	-	2	2	- 38	Normal	
32	10.0 per cent	$\frac{1}{4}$	+	9+	8	+ 56		
34	10.0 per cent	5	+	14+	13	+ 50		
35	1.0 per cent	5	+	$3\frac{1}{2}$	1	+ 20	Normal	
35	10.0 per cent	5	+	11+	2	+100		
41	1.0 per cent	$\frac{1}{2}$	+	27	$12\frac{1}{2}$	+ 43		
41	1.0 per cent	1	+	13	1	+116	Normal	

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE + = INCREASE	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECT	REMARKS
Cotarnine phthalate (styptol)								
32	0.1 per cent	minutes 1	+	minutes 12	minutes 7	per cent + 30	Normal	
42	0.1 per cent	$\frac{1}{2}$	+	20	Immediate	+320		
Heat								
55	38°C.	1	+	7	Immediate	+ 81	Normal	
Alum								
42	10.0 per cent	$\frac{3}{4}$	+	10	2	+420	Normal	
	10.0 per cent	5	+	8	2	+110	Normal	
54	5.0 per cent	5	+	5	1	+ 30	Normal	
51	1.0 per cent	5	+	24+	16	+ 55		
32	10.0 per cent	5	Unchanged in 15 minutes					
Papaverin hydrochloride								
42	1.0 per cent	1	+	22	$\frac{1}{2}$	+180	Normal	
	0.1 per cent	$\frac{1}{2}$	+	25	$\frac{1}{2}$	+120	Normal	
55	0.1 per cent	5	+	12	1	+ 40		
Chelidonin sulphate								
42	0.1 per cent	$\frac{1}{2}$	+	5	$\frac{1}{2}$	+ 56	Normal	
61	0.1 per cent	5	+	6	1	+115	Normal	
Fluid extract hydrastis (alcohol free)								
43	100.0 per cent	5	-	21	3	- 55	Normal	
44	100.0 per cent	1	+	18	1	+ 77		
	100.0 per cent	5	+	17	1	+100		
46	100.0 per cent	5	+	19	1	+ 31		

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (— = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (—) OR INCREASE (+)	AFTER EFFECT	REMARKS
Fluid extract ergot (alcohol free)								
		minutes		minutes	minutes	per cent		
44	100.0 per cent	1	+	17	2	+ 63	Normal	
	100.0 per cent	5	+	11	1	+125	Normal	
45	100.0 per cent	5	+	10	1	+174	Normal	
Apothesine								
53	1.0 per cent	5	—	20+	20	— 41		
52	1.0 per cent	5	+	21+	21	+ 33		
58	1.0 per cent	5	+	8	1	+ 31	Normal	
Sodium nitrite								
60	1.0 per cent	5	+	10	$\frac{1}{2}$	+ 55	Normal	
48	5.0 per cent	5	+	8	1	+ 60	Normal	
61	5.0 per cent	5	+	16	1	+267	Normal	
53	1.0 per cent	5	+	10	$\frac{1}{2}$	+ 88	Normal	
Antipyrin								
48	10.0 per cent	5	+	15	$\frac{1}{2}$	+ 80	Normal	
53	10.0 per cent	5	+	5	$\frac{1}{2}$	+ 50	Normal	
Novocain								
52	1.0 per cent	5	+	15	15	+ 82		
53	1.0 per cent	5	+	15	15	+ 60		
56	1.0 per cent	5	+	15	1	+ 60	Normal	
Berberin								
54	Saturated	5	+	11	6	+ 10	Normal	
54	Saturated	5	+	6	$\frac{1}{2}$	+ 26	Normal	
Narcotin								
55	0.1 per cent	5	+	30	15	+ 53	Normal	
60	0.1 per cent	5	+	6	$\frac{1}{2}$	+ 30		

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (- = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (-) OR INCREASE (+)	AFTER EFFECT	REMARKS
Stovain								
56	1.0 per cent	5 minutes	+	6 minutes	$\frac{1}{2}$ minutes	+120 per cent	Normal	
Orthoform								
57	1.0 per cent	5	+	1	1	+ 16	Normal	
56	1.0 per cent	5	+	13	1	+177		
	1.0 per cent	5	+	15	1	+136		
Emetin hydrochloride								
56	Saturated	5	+	10	1	+100	Normal	
	Saturated	5	+	11	2	+ 31	Normal	
Peptone								
56	5.0 per cent	5	+	11	6	+ 38	Normal	
58	5.0 per cent	5	+	10	6	+ 9	Normal	
C. Hemorrhage unchanged or variable								
Potassium permanganate								
61	2.0 per cent	5	-	11+	5	- 30	Normal	
51	1.0 per cent	5	+	11	1	+ 60	Normal	
Lactose								
61	10.0 per cent	5	-	5	2	- 50	Normal	
54	5.0 per cent	5	Unchanged in 20 minutes					
57	5.0 per cent	5	Unchanged in 15 minutes					
Coagulen (Ciba)								
49	1.0 per cent	5	-	15	10	- 11	In N.S. In N.S.	
49	1.0 per cent	5	-	6	2	- 45		
49	1.0 per cent	5	-	5	1	- 20		

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (— = DECREASE, + = INCREASE)	DURATION OF EFFECT	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (—) OR INCREASE (+)	AFTER EFFECT	REMARKS
Coagulen (Ciba)—Continued								
		minutes		minutes	minutes	per cent		
50	5.0 per cent	5	—	3	1	— 18		
50	5.0 per cent	5	+	11	6	+ 60	Normal	
50	10.0 per cent	5	+	20+	6	+ 54	Normal	
50	10.0 per cent	5	+	21	11	+ 54	Normal	
Atropine sulphate								
51	0.1 per cent	5	—	7	2	— 50	Normal	
61	0.1 per cent	5	+	16	11	+117	Normal	
Gelatin								
57	5.0 per cent	5	{ First+ Later—	15	10	+ 34	Normal	
				13	6	— 40		
58	5.0 per cent	5	+	13	6	+ 76		
Camphor								
47	Saturated	5	—	16+	16	— 75	Normal	
48	Saturated	5	+	15	1	+ 50		
Histamin								
47	0.1 per cent	3	{ First+ Later—	8	3	+ 25	Normal	
				23+	10	— 58		
51	0.1 per cent	5	{ First— Later+	8	1	— 60		
				22	15	+363		
52	0.1 per cent	5	—	8	$\frac{1}{2}$	— 47	Normal	
Thromboplastin (Squibb)								
54	Whole	5	+	5	$\frac{1}{2}$	+ 90	Normal	
55	Whole	5	—	21	11	— 60	Normal	
Sparteïn								
51	1.0 per cent	5	Unchanged in 20 minutes					

TABLE 1—Continued

NUMBER OF EXPERIMENT	CONCENTRATION USED	DURATION OF IRRIGATION	EFFECT ON BLEEDING (— = DECREASE, + = INCREASE)	DURATION OF EFFECTS	TIME OF APPEARANCE OF MAXIMAL EFFECT	MAXIMAL DECREASE (—) OR INCREASE (+)	AFTER EFFECT	REMARKS
Menthol								
56	Saturated	5	Unchanged in 30 minutes					
57	Saturated	5	Unchanged in 15 minutes					
Tincture veratrum album (alcohol-free)								
		<i>min-utes</i>		<i>min-utes</i>	<i>minutes</i>	<i>per cent</i>		
46	10.0 per cent	5	Unchanged in 20 minutes					
Tincture aconite (alcohol-free)								
46	10.0 per cent	5	Unchanged in 20 minutes					
Kephalin (fresh: Howell's method)								
62	5.0 per cent	5 {	First— Later+	2 5	1 2	— 25 + 15	+	In 1 per cent citrate
62	2.5 per cent	5 {	First— Later+	3 10	2 3	— 32 + 58	Normal	In 0.5 per cent citrate
62	0.5 per cent	5 {	First— Later+	3 10	1 3	— 20 + 30	Normal	In 1 per cent citrate
62	5.0 per cent	10	—	28	2	— 45	Normal	In 1 per cent citrate
Kephalin (Armour)								
49	0.2 per cent	5	+	13+	2½	+210	Normal	In N.S.
50	0.2 per cent	5	+	12	2½	+ 50	Normal	
50	0.2 per cent	5	+	26	1	+ 33	Normal	
56	2.0 per cent	5	+	11	½	+ 71		
33	5.0 per cent	1	—		15	—100		

It is interesting to note that the hemorrhage is almost invariably increased after the hemostatic effect disappears. This is apt to occur quite suddenly after a pronounced reduction in hemorrhage, and seems

TABLE 2

Classification of various local agents on hemorrhage from the dog's foot-pad

DIMINISHED	INCREASED	UNCHANGED OR VARIABLE
Acetic acid	Alum	Aconite*
Atropin	Antipyrin	Camphor
Barium chloride	Apothesine	Coagulen (Ciba)
Caffein	Beef serum	Gelatin
Calcium chloride	Berberin	Histamin
Cane-sugar	Chelidonin	Kephalin
Cocain	Chloral	Lactose
Digitalis	Chlorazene	Menthol
Epinephrin (1: 1,000,000 and higher)	Dichloramin-T	Permanganate
Ferric chloride	Emetin	Sparteин*
Glycerite of tannic acid	Epinephrin (1: 10,000,000 to 50,000,000)	Thromboplastin (Squibb)
Hydrochloric acid	Ergot, fluid extract	Veratrum*
Morphin	Horse serum	
Normal saline	Hydrastis, fluid extract	
Peptone, 10 per cent	Hypochlorite solution (Dakin's)	
Pituitary extract	Hydrastinin hydrochloride	
Quinin	Narcotin	
Quinin-urea hydrochloride	Nitrite	
Silver nitrate*	Novocain	
Sodium bicarbonate	Orthoform	
Sodium chloride, 10 per cent	Papaverin	
Strophanthus	Peptone, 5 per cent	
Tannin	Salicylate*	
Turpentine	Stovain	
Tyramin	Stypticin	
Zinc sulphate	Styptol	

* Only one trial was made with these drugs. Their position may change with more experiments.

to be associated with low concentrations of the drug in the wound tissue. This is supported by the fact that the only demonstrable effect of very low concentrations was an increase in hemorrhage. The results in table 1 show that the concentration of 1: 10,000,000 increased the

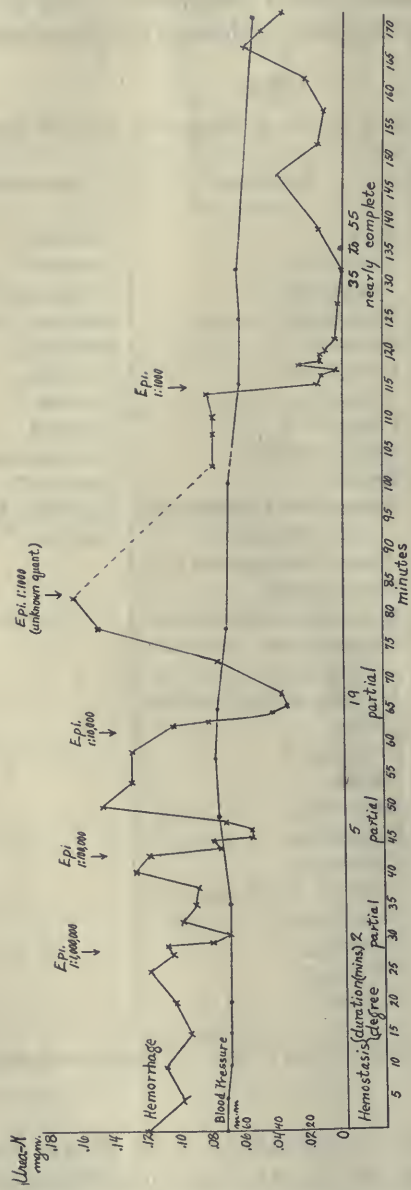


FIG. 2. EXPERIMENT 36. LOCAL EFFECT OF EPINEPHRIN IN DIFFERENT CONCENTRATIONS ON THE COURSE OF HEMORRHAGE FROM THE DOG'S FOOT-PAD

Illustrating the relation between concentrations ranging from 1:1,000,000 to 1:1000 on the degree and duration of hemostasis. "Epi." means epinephrin. 10 cc. of the solution was applied for 1 minute in each case.

bleeding, and the increase was greater with 1:50,000,000. The effects were almost immediate, reaching their maximum in $2\frac{1}{2}$ to 3 minutes and lasting 4 to 14 minutes with irrigations of 1 minute. The action of the epinephrine here is undoubtedly on the blood vessels in the wound, and it is strictly local without involvement of the central nervous system or different vascular areas, such as the skeletal musculature, mechanisms, which have been invoked with the systemic administration of epinephrin (Hartmann (5); Gruber (6)). It is certain that vasodilation produced by epinephrine is not peculiar to systemic administration.

The same phenomenon has been reported clinically. That is, in the application of epinephrin to wounds an increase in bleeding has been observed as a late change. Proof of this is seen in the results here reported, and accordingly epinephrine is not a dependable hemostatic.

This phenomenon is not limited to epinephrine, for it was observed in some experiments with cocain (also a sympathicomimetic agent), which at first reduced the hemorrhage presumably due to vasoconstriction. In other experiments the only effect of cocain observed was an increase in bleeding. The phenomenon was not observed with tyramin or histamin. Increased bleeding produced by other agents was due to other causes.

Tyramin. In concentrations ranging from 0.1 to 0.5 per cent tyramin rather promptly, but also temporarily, diminished the bleeding by about 65 per cent. The effect takes somewhat longer to appear and is not so lasting as with epinephrin. There was no tendency to increase the bleeding after the initial hemostasis in which it differs from epinephrin. As a hemostatic, tyramin, according to this, is inferior to epinephrin, but is not entirely devoid of hemostatic properties as seems to be implied in a recent statement of Hewlett's (7).

Pituitary extract. Specimens from three different sources were used and they generally tended to exhibit the same phenomena. The initial effect consists of a momentary (for about $\frac{1}{2}$ minute) increase in bleeding followed by a more or less prolonged decrease, though never a permanent arrest. The time of appearance of the maximum hemostatic effect, and the duration were rather variable, that is, 1 to 14 and 3 to 17 minutes, respectively. The diminution in bleeding also varied considerably, that is, from 24 to 77 per cent of the original level. In 2 out of 9 experiments

there was an increase in hemorrhage, and, this, as well as the initial increase in the majority of the experiments, may be attributed to the reaction of the pituitary extracts some of which were rather markedly acid, which in low concentrations is known to produce vascular relaxation. The blood on the wounds and in the wash-fluids was distinctly dark due to the formation of acid hematin. On the whole, pituitary extract is inferior and unreliable as a hemostatic, but does not increase bleeding as an after effect.

Histamin. The ordinary systemic actions of histamin are generally produced by very low concentrations, but the application of a relatively high concentration (0.1 per cent) to wounds gave extremely variable and comparatively unimportant effects on bleeding. This drug may be dismissed without further consideration.

Digitalis group and allies

The effects of digitalis, strophanthus, sparteine, barium chloride and caffeine were studied.

Digitalis and strophanthus. Irrigations of the wounds with alcohol-free tinctures rather slowly (in 5 to 30 minutes) and temporarily (for 3 to 30 minutes) and moderately (by 19 to 86 per cent, diminished the bleeding. Qualitatively this seems to agree with their constrictor action on the blood vessels when administered (in high doses) systemically.

Sparteine. Irrigations with a 1 per cent solution of sparteine sulphate for 5 minutes did not alter the course of hemorrhage in 20 minutes.

Barium chloride. Irrigations with 1 and 10 per cent solutions of barium chloride for $\frac{1}{4}$ to 5 minutes lessened local bleeding by 10 to 100 per cent in 1 to 10 minutes for 1 and 60 minutes in 3 experiments. The effects appear to be proportional to the duration of irrigation, and presumably due to vasoconstriction. It is seen that one 5 minute irrigation (Expt. 46) produced a lengthy and complete hemostasis.

Caffein. Two irrigations of two different wounds with a 1 per cent solution of caffein for 5 minutes each reduced bleeding by 33

to 82 per cent in 1 to 10 minutes and this lasted for 3 to 20 minutes. The bleeding then returned to the previous level as was nearly always the case with the different members of this group.

It may be concluded that digitalis, strophanthus, sparteine and caffein are distinctly inferior as hemostatics; digitalis and strophanthus would be undesirable because of their irritating properties. Barium is a prompt and efficient hemostatic, but whether or not because of its systemic toxicity it would be clinically promising is not now known.

Local anesthetics

Cocain. The effects of cocain in different concentrations (1 to 10 per cent) were variable, but in the majority of experiments the bleeding was decreased by 43 to 100 per cent, followed by an increase. The maximum effect appeared on an average in about $3\frac{1}{2}$ minutes and lasted about 10 minutes, which does not compare favorably with epinephrin.

Novocain. Three 5-minute irrigations with a 1 per cent solution increased bleeding from 60 to 82 per cent in 1 to 15 minutes and this lasted in each case for 15 minutes. The bleeding returned promptly to the previous level.

Stovain. Owing to the shortage of the drug only one 5-minute application was made. This increased bleeding by 120 per cent in $\frac{1}{2}$ minute and the effect lasted for 6 minutes.

Orthoform. One per cent solutions of the drug (at 38°C.) were used. Three irrigations for 5 minutes each increased bleeding from 16 to 177 per cent in 1 minute and the effects lasted for 13 to 15 minutes.

Antipyrin. In high concentration this drug has been advised as a hemostatic. Two 5-minute applications of a 10 per cent solution increased the bleeding from 5 to 80 per cent in $\frac{1}{2}$ minute and the effects lasted 5 to 15 minutes, respectively. The bleeding then returned to the previous level.

Apothesine. With this drug the effects were moderate and somewhat variable. Two irrigations for 5 minutes each with a 1 per cent solution increased the bleeding by 31 and 33 per cent in 1 and 21 minutes and the effects lasted 18 and 21 min-

utes, respectively. With one other application bleeding was lessened by 41 per cent in 20 minutes and the effect lasted for more than 20 minutes.

Quinine-urea hydrochloride. Three irrigations with a 1 per cent solution for 5 minutes each reduced bleeding by 36 to 70 per cent in 5 to 15 minutes and the effects lasted for 15 to 20 minutes. One-tenth per cent quinine affected bleeding in the same direction.

The effects of cocain and the various substitutes tested generally agree with the reported vascular effects. Contrary to the reports, antipyrin does not possess hemostatic qualities, but rather increases bleeding. The effects obtained with orthoform, although used in rather high concentration considering the solubility of the drug, which, however, dissolves to 1 per cent concentration at body temperature, are of special importance in connection with its use for the relief of pain in gastric ulcer and similar lesions. That is, its use could conceivably lead to hemorrhage of variable proportions with its undesirable consequences. This would also tend to lessen its activity by dilution and continued removal. Not much is known as yet about apothesine, but it appears to be less active than some of the other anesthetics tested. That is, the weak vascular effects harmonize with its low efficiency as a local anesthetic as observed by Sollmann (8).

Astringents

The astringents tried were ferric chloride (1 per cent), zinc sulphate (5 per cent), alum (1 and 10 per cent), silver nitrate (1 per cent), tannin (1 and 5 per cent) and the glycerite of tannic acid (U.S.P.).

Ferric chloride. This was more constant, but somewhat less effective than tannin. Bleeding was promptly arrested when the vessels involved were not too large. As a rule the wounds after treatment with high concentrations of this astringent were no longer utilizable for the application of other drugs. The normal tissue was hardened, but frequently the bleeding continued, though lessened. Three irrigations with the 1 per cent solution

from 1 to 5 minutes lessened bleeding by 22 to 100 per cent in 1 to 15 minutes and the effect lasted 6 to 40 minutes.

Alum. Bleeding was increased from 30 to 420 per cent by alum in concentrations ranging from 1 to 10 per cent, the maximum effect being reached in 8 to 16 minutes, and lasting from 5 to 24 minutes. In general the higher the concentration the greater the increase in the hemorrhage. The wound did not show the presence of coagulated blood as with iron and tannin and the wash-fluids precipitated only after standing for some time.

It is conceivable that the presence of citrate interfered with any hemostatic action of alum. However, if this is due to the precipitation of protein it cannot be attributed to citrate with the 5 and 10 per cent solutions, for precipitation of dog serum was more effectively produced in the presence of citrate than in the plain alum solutions. The precipitation of serum by 1 per cent alum containing 1 per cent citrate was retarded, but not permanently inhibited. Results illustrating the behavior of alum and other metallic salts on the precipitation of dog serum are appended in table 3.

The acidity of the high concentrations of alum is probably responsible for the absence of precipitation in the solutions without citrate. The presence of citrate diminishes the acidity to an optimal point, hence the greater precipitation in the alum solutions containing small quantities of citrate. From this it is not clear why alum (in citrate) does not exert the ordinary actions of an astringent when it comes in contact with wounds. The increase in bleeding observed is perhaps due to the acidity, which in low concentrations is known to produce vascular relaxation. The common practice of using alum in sticks is a different matter in which the factor of pressure may be the chief element responsible for the hemostasis.

The results with ferric chloride indicate that precipitation is beneficially affected in the presence of citrate. Zinc sulphate is uninfluenced.

Tannin. With concentrations of 1 and 5 per cent the results were more constant, but in the form of the glycerite (20 per cent tannin) tannin gave variable results. Taking the glycerite prep-

aration, an equal number (2) of experiments showed a decrease from 60 to 100 per cent, and an increase from 110 to 370 per cent

TABLE 3

Effect of citrate on the precipitation of dog serum by certain metallic salts

METALLIC SALT	CONCENTRATION USED	CONDITION	PRECIPITATION
	<i>per cent</i>		
Alum.....	10	Without citrate	No precipitation in 30 minutes
	10	With citrate (1 per cent)	Incomplete precipitation in 15 minutes
	5	Without citrate	Incomplete precipitation end of 13 minutes
	5	With citrate (0.5 per cent)	Complete precipitation in 9 minutes
	5	Without citrate	Complete precipitation in 9 minutes
	5	With citrate (1 per cent)	Complete precipitation in 1 minute
	1	Without citrate	Complete precipitation in 3 minutes
	1	With citrate (1 per cent)	Discernible precipitation in 10 minutes Incomplete precipitation in 30 minutes
Ferric chloride.....	5	Without citrate	Complete precipitation in 5 minutes Less complete precipitation in 15 minutes
	5	With citrate (1 per cent)	Complete precipitation in 5 minutes Remains complete precipitation in 15 minutes
Zinc sulphate.....	5	Without citrate	No precipitation in 27 minutes
	5	With citrate (1 per cent)	No precipitation in 26 minutes

in the bleeding from different wounds. With the aqueous solution of 5 per cent strength and irrigations for 5 minutes each, bleeding was practically arrested (reduced by 95 to 100 per cent) in

1 and 5 minutes and the arrest was practically permanent, not being observed beyond 15 and 30 minutes, respectively. Irrigations with the 1 per cent solution for 5 minutes lessened bleeding by 67 per cent in 8 minutes and the effect lasted for 15 minutes, then the bleeding returned to the previous level. In another experiment irrigation with a 1 per cent solution for 1 minute increased bleeding by 66 per cent in 7 minutes and the effect lasted for 12 minutes, then returned to the previous level. On the whole, therefore, aqueous solutions of tannin lessen or arrest bleeding more efficiently than the glycerite.

Precipitation of blood and wound tissue and wash-fluids was observed in all of the experiments, but the bleeding continued underneath the coagula. The act of irrigation was not responsible for the continued bleeding, for irrigation with normal saline under the same conditions allowed the formation of a clot and bleeding stopped promptly (in 10 to 15 minutes).

Similar effects were observed with both the glycerite and aqueous solutions of tannin with unirrigated wounds of the cat's pad described in a previous publication. The glycerite was also found unsatisfactory as a hemostatic with oozing wounds of the diploï and lacerated wounds of the head of the cat.

Silver nitrate. Only one experiment was performed, and in this a 5-minute irrigation with 1 per cent silver nitrate reduced bleeding by 75 per cent in 3 minutes and the effect lasted for 15 minutes, the bleeding continuing at the end of this time.

Zinc sulphate. Two irrigations were made for 5 minutes each. Bleeding was diminished by 14 and 53 per cent in 1 and 6 minutes and the effects lasted for 7 and 10 minutes, respectively. The bleedings then returned to their previous levels.

It is interesting to note the relation of the wound coagula to the bleeding. Alum and zinc sulphate, even in the highest concentrations, did not coagulate the blood on the wounds. On the other hand, ferric chloride, tannin and silver nitrate produced prompt and marked coagula which accumulated on the wound in large masses and appeared to seal the wound entirely. The wash-fluid continued to be tinged with blood, and the act of continuous washing with citrate might be held responsible for this, but, as

pointed out above, washing under the same conditions with saline permits the formation of natural clots with arrest of bleeding rather promptly. The coagula stuck loosely, were easily detached and oozing blood was revealed. Exactly the same phenomena were observed in experiments previously reported in which these agents were applied directly to the wounds without further interference. In other words, the proteins of the blood and wound tissues are promptly coagulated by these astringents wherever they come in contact with them, the penetration of the astringent being limited by the thick coagulum and the seepage of blood from underneath continues. This together with the infirmity of the clots renders the efficacy of these agents rather dubious.

From all this it appears that the astringents as a class are not highly efficient and reliable as local hemostatics. It is generally admitted that iron salts although quite effective, are undesirable because of their irritating qualities.

Osmotic agents

Sodium chloride (0.9 to 10 per cent), sodium bicarbonate (2 per cent), acetic (0.1 per cent) and hydrochloric acids (1 per cent) lactose (5 per cent) and cane sugar (10 per cent) were tested. Strong salt solution and vinegar are common lay remedies for bleeding. Bastedo (9) mentions acetic acid as a local hemostatic, and according to Morrihy (10) cane-sugar has been used by Sammarlina both as a hemostatic and antiseptic.

Sodium chloride. Four irrigations for 5 minutes each with a 0.9 per cent solution without citrate practically arrested hemorrhage (i.e., 90 to 100 per cent) in 1 to 5 minutes after the irrigation was completed and the effects remained permanent at the end of 10 to 15 minutes. In another experiment with some disturbance in blood pressure bleeding was reduced by only 50 per cent. The effects with normal saline containing citrate were in the same direction, but not so pronounced as would be expected. That is, two irrigations for 5 minutes each lessened bleeding by 35 and 38 per cent in 1 and 10 minutes and the effects lasted for 11 and 15 minutes, respectively.

The 10 per cent solution with citrate did not seem to be any more effective than the 0.9 per cent. With two irrigations for 5 minutes each bleeding was reduced by 33 to 45 per cent immediately after the irrigation was completed and the effects lasted for 11 to 20 minutes. Immediately on application of the different concentrations, except plain normal saline, the wounds showed the presence of small white punctate areas presumably due to dehydration of small clots of fibrin or shreds of other tissue by the high molecular concentration of the solutions. There was also a momentary increase in the effusion of fluid (chiefly blood) in the beginning, and for the same reason.

Acids. Both the acetic and hydrochloric acids reduced bleeding quite markedly, that is, from 53 to 84 per cent. The effects required about 10 minutes to develop and were lasting, the wounds, as a rule, being useless for further experimentation, although bleeding was not completely arrested. The wound tissues appeared rather anemic, and were leather-like to the touch. Constriction of vessels in the frog's mesentery by strong acids on direct application, was noted by I. Adler (11).

Bicarbonate. The 1 per cent solution with two irrigations was not so effective an hemostatic as a corresponding strength of hydrochloric acid used. Bleeding was lessened by about 33 to 40 per cent in 1 and 10 minutes and the effects lasted for about 6 and 15 minutes, respectively. This is of some interest in connection with the use of alkaline washes for wounds, such as Dakin's hypochlorite solution and bicarbonate borate mouth washes. According to some (12) Dakin's solution increases bleeding from wounds. My results indicate that alkalinity is not an important contributing factor in this. This also agrees with recent observations reported by Meltzer and Githens (13). The results also agree with those of I. Adler (11) on the mesenteric vessels of the frog in which direct application of bicarbonate caused constriction of the vessels with slowing of the blood stream.

Sugars. Of the two sugars used (lactose and saccharose), only the 10 per cent solutions of lactose and cane-sugar diminished hemorrhage. The effects of two 5-minute applications of cane sugar (10 per cent) appeared rather promptly (in 2 minutes) and

lasted for about 12 minutes, and the bleeding was diminished by 40 to 60 per cent. Two irrigations with 5 per cent lactose for 5 minutes each did not change the course of bleeding in 15 and 20 minutes. Ten per cent lactose reduced bleeding by 50 per cent in 2 minutes, and the effect lasted for 5 minutes.

The effects of the two sugars (10 per cent concentrations) can be explained on an osmotic basis, for both solutions were hypertonic. The results with lactose are of interest in connection with the experiments with coagulen, which contains about 5 per cent lactose.

It may be concluded that the application of 0.1 per cent acetic and 1 per cent hydrochloric acids (relatively high degrees of acidity), 10 per cent solutions of cane-sugar, lactose, and sodium chloride more markedly, and 1 per cent bicarbonate somewhat less effectively diminish local hemorrhage. The reported hemostatic effects of strong salt and sugar solutions, and acetic acid have been confirmed.

Opiates and cotarnine

The following were studied: Morphin, narcotin, papaverin, chelidonin, and also cotarnine hydrochloride (stypticin) and cotarnine phthalate (styptol), which are derivatives of narcotine. The cotarnine salts have been used and there are extensive claims made for them as hemostatics both local and systemic. Cotarnine and the remaining agents are of interest in connection with the relation of chemical structure to the pharmacological action of the alkaloids of opium.

Morphin. Local hemorrhage was promptly diminished by morphin (0.1 to 1 per cent) with applications of 5 minutes each for about 15 minutes and to the extent of about 55 per cent of the original level.

Narcotin. A 0.1 per cent solution increased bleeding by 53 and 30 per cent, and this lasted for 30 and 6 minutes, respectively, with applications for 5 minutes each.

Papaverin. Two applications with a 0.1 per cent solution for $\frac{1}{2}$ and 5 minutes increased bleeding by 120 and 40 per cent and the effects lasted for 25 and 12 minutes, respectively. A 1 per

cent solution applied for 1 minute, increased bleeding by 180 per cent in $\frac{1}{2}$ minute and the effect lasted for 22 minutes.

Chelidonin. The application of a 0.1 per cent solution for $\frac{1}{2}$ and 5 minutes increased bleeding by 56 and 115 per cent in $\frac{1}{2}$ and 1 minute, and the effects lasted for 5 and 6 minutes, respectively.

Cotarnine. Ten experiments with cotarnine hydrochloride (stypticin) were made on seven different wounds. The concentrations ranged from 1 to 10 per cent, and the duration of application varied from $\frac{1}{4}$ to 5 minutes. The hemorrhage was invariably increased, ranging from 20 to 218 per cent above the previous level, and the effects lasted from 2 to 27 minutes. The results from two experiments with 0.1 per cent cotarnine phthalate (styptol) were in the same direction. That is, an increase in hemorrhage by 30 and 320 per cent with applications of 1 and $\frac{1}{2}$ minute occurred and the effects lasted for 12 and 20 minutes, respectively.

The results obtained with the different alkaloids of opium agree with the classification (14) of these according to the relation of their chemical structure to pharmacological actions on different organs (uterus, blood vessels, bronchi, intestine and stomach, seminal vesicles and ureter). That is, morphine (phenanthrene derivative) diminished hemorrhage presumably by vasoconstriction, and narcotin, papaverin, chelidonin (isoquinolin derivatives) and also cotarnine (narcotin derivative) increased bleeding presumably by vascular relaxation from depression of smooth muscle. The results with stypticin and styptol (cotarnine salts) are confirmative of experiments on bleeding reported in a previous publication, and prove conclusively that these products are totally devoid of hemostatic properties and the claims made for them are exaggerated and unjustified. In fact, they are detrimental, since they increase bleeding if anything.

An opportunity presented itself to make a few observations with stypticin, alum and epinephrin on a superficial wound in a human subject. The results were as follows:

Subject P. J. H.: Superficial wound of thumb about 1 cm. square and involving papillary layer of skin. The application of 5 per cent styp-

ticin increased the bleeding in 2 minutes and this continued for 10 minutes; the bleeding was further increased by massaging and exercising the arm. 10 per cent alum was now applied; pain was felt and bleeding was somewhat diminished, but there was no complete arrest; the citrate irrigation fluid remained tinged red; massaging and exercising the limb increased the bleeding. 1:1000 epinephrin was now applied; diminution in bleeding was noticeable in 15 seconds and complete arrest occurred within $\frac{1}{2}$ minute after application; irrigation with citrate remained colorless; massaging of thumb and exercising the limb did not induce bleeding; duration of arrest was 10 minutes; at end of 10 minutes oozing of blood began. The wound was pale white and pain was felt for sometime after irrigation with epinephrin, i.e., during the hemostasis, presumably local pain was accentuated by the anemia. Treatment with epinephrin was repeated with similar results, namely, bleeding was diminished in 15 seconds and completely arrested in 1 minute; duration of arrest was about 10 minutes. The application of 5 per cent stypticin started bleeding in 2 minutes and this continued to increase markedly at the end of 5 minutes. A sharp stinging pain of momentary duration was felt when stypticin was applied. 1:1000 epinephrin was now applied again, resulting in complete arrest of the bleeding in $1\frac{1}{2}$ minutes, and 4 minutes later oozing of blood was again resumed, suggesting that the epinephrin is not effective after previous treatment of the wound with stypticin. No sensation of pain was felt at this time.

These results confirm the hemostatic qualities of epinephrin observed on dog wounds, and the practical worthlessness of stypticin and alum as local hemostatics.

Thromboplastic agents

The following were tested; kephalin, coagulen and thromboplastin. Experiments with horse and beef serums are also included.

Kephalin. Four irrigations of pad-wounds for 5 minutes each with emulsions of kephalin (Armour's) ranging from 0.2 to 2 per cent in citrate and normal saline increased the hemorrhage from 33 to 210 per cent in $\frac{1}{2}$ to $2\frac{1}{2}$ minutes and the effects lasted for 11 to 26 minutes. Irrigation for one minute with a 5 per cent emulsion in one experiment completely arrested the hemorrhage in 15 minutes. All of these emulsions were made by first dis-

solving the kephalin in ether (usually a 5 per cent solution) and from this the dilutions were made, removing the ether by aeration before applying the emulsion. It is possible that the 5 per cent emulsion in this experiment contained some ether which by cooling contributed to the arrest of hemorrhage.¹

Further experiments were made with a product freshly prepared from six pigs' brains and according to the method of Howell (15) as described by McLean. The product was used next day after the method of preparation was completed. Four irrigations of the same wound with emulsions in citrate ranging from 0.5 to 5 per cent were made. In one experiment the concentration of citrate in the citrate irrigation fluid was reduced by one-half (i.e., 0.5 per cent) as I was informed by Professor Howell² that the 1 per cent concentration might interfere with the thromboplastic activity of the product, while lower concentrations would appear to facilitate this process. The results with three irrigations were quite uniform and as follows: The strength of the emulsions ranged from 0.5 to 5 per cent. In one experiment 0.5 per cent citrate was used; in the others 1 per cent. In all these experiments the bleeding was momentarily lessened, then increased for a longer period, that is, bleeding was diminished by 20 to 32 per cent in 1 to 2 minutes and the effects lasted for 2 to 3 minutes. Later, bleeding was increased by 15 to 58 per cent in 2 to 3 minutes and the effects lasted for 5 to 10 minutes. The bleeding in each case returned to the previous level.

One irrigation with a 5 per cent emulsion in 1 per cent citrate for 10 minutes was made. Bleeding was reduced by 45 per cent in 2 minutes and the effect lasted for 28 minutes. There seemed to be a thin, reddish membranous layer on the wound, but bleeding continued.

¹ The hemostatic qualities of ether were not specially studied in my experiments, but I have occasionally noticed that irrigation with saline containing ether stopped superficial bleeding. While this paper was in press, a contribution by Moorhead (*Journ. Am. Med. Assoc.*, 1918, 17; 738) reports the successful use of ether as a hemostatic, also antiseptic, in joint and superficial injuries in the war zone in France.

The concentrations of kephalin used in these experiments exceed those generally recommended for use in the treatment of hemorrhage. Nevertheless, the results (under the conditions) do not agree with the optimistic reports of others with kephalin and extracts of brain and other tissues whose action is said to depend on kephalin or other phosphatid-like constituents. Hirschfelder (16) reports favorable effects from brain extract by application of the kephalin containing residue to an incised femoral artery bleeding into Scarpa's triangle (dog), and also in other regions (diploi, kidney, liver, teeth and tonsils). Cecil (17) claims favorable hemostatic effects from gauze impregnated with kephalin and packed with some pressure in operations on the prostate, but denies the practical value of kephalin smears and kephalinated gauze for other wounds from which it appears that pressure is an important contributing factor in the successful use of kephalinated gauze. Hurwitz and Lucas (18) report early arrest of hemorrhage in hemophiliacs with local application of kephalin. The use of controls in all these optimistic experiments and reports seems inadequate in view of the numerous factors which are known to contribute to the normal coagulation of blood and arrest of hemorrhage. In some of my experiments with kephalin very small clots of fibrin near by or over the mouths of the incised blood vessels were seen, but the oozing of blood continued. No clots at all appeared on other wounds. It is quite possible that with small punctate wounds, such for instance as are used in Duke's method of testing bleeding time, the application of kephalin facilitates hemostasis, but with larger wounds the flow of blood is more vigorous and voluminous, resulting in removal and dilution of the agent applied. This is mitigated by the continuous irrigation in my method. On the other hand, the continuous irrigation might be regarded as disturbing to adequate clot formation, but this is certainly not the case with normal saline and with such coagulants as ferric chloride and tannin. What effect the citrate has on the action of kephalin is not definitely known although it does not seem that calcium deprivation or inhibition interferes with its thromboplastic activity. For, according to the work of

Howell (14) and his pupils, this activity (in vitro) is not interfered with in oxalated (calcium-free) plasma.

Coagulen (Ciba).⁴ This is said to contain substances which exist naturally in blood and hematopoietic organs and are apt to accelerate the coagulation of blood. It also contains about 5 per cent of lactose. Its use is advised in all kinds of hemorrhages, although it is claimed to be chiefly efficacious when applied directly to the spot. Its use by all the known methods of administering drugs is recommended.

Precautions were taken to apply coagulen according to directions in the manufacturer's circular. Different concentrations ranging from 1 to 10 per cent in saline and citrate were previously heated and applied to the dog's pad at body temperature (38°C.) for variable periods of time not exceeding 5 minutes. The effects were then compared with irrigations of saline and citrate alone and also lactose (5 per cent). In this way some idea of the value of the product could be gained.

Coagulen with citrate. In one experiment with an irrigation of a 1 per cent solution for 5 minutes, bleeding was reduced by 11 per cent in 10 minutes and the effect lasted for 15 minutes. Two irrigations were made with a 5 per cent solution for 5 minutes each. With one bleeding was reduced by 18 per cent in 1 minute and the effect lasted 3 minutes. In the other experiment the bleeding was increased by 60 per cent in 6 minutes and the effect lasted for 11 minutes. Two irrigations for 5 minutes each were made with a 10 per cent solution. Both increased the bleeding by 54 per cent in 6 and 11 minutes and the effects lasted for about 20 minutes.

Coagulen in normal saline (without citrate). Two irrigations with a 1 per cent solution were made for 5 minutes each. Bleeding was reduced by 20 and 45 per cent in 1 and 2 minutes and the effects lasted for 5 and 6 minutes, respectively. However, irrigations with saline alone (without coagulen and citrate) in experiments 49, 50 and 55 gave much better results. The presence of coagulen, therefore, does not contribute to the hemostasis.

⁴ Supplied by A. Klipstein and Company.

Small clots were present on the wounds in all of the experiments made with saline alone and coagulen in saline and citrate, and appeared equally promptly with all solutions, but the bleeding continued. The presence of lactose does not contribute anything to the effects, for irrigation with 5 per cent solutions under the same conditions did not alter the course of bleeding.

It does not appear, therefore, that coagulen under the conditions possesses hemostatic properties.

Thromboplastin (Squibb).⁵ This is made from brain tissue of the ox and is said to contain kephalin. Like coagulen it is advised for hemorrhage due to any cause whatsoever, from small veins and capillaries, in surgical operations for adenoids, in the nose and throat, rectum, into the gums following extraction of teeth, hepatic bleeding, and "in cases of true hemophilia it may be regarded almost as a specific hemostatic." The clinical reports are vague and uncontrolled. Clinical cases are reported (in the manufacturer's literature) in which hemostasis seemed to be favored after failure to check bleeding by the application of epinephrin, tannin, silver nitrate, etc. The results of my experiments deny any position of importance to thromboplastin as compared with these agents.

The results from two experiments on the wounds of two different animals in which thromboplastin (with and without citrate) was applied for 5 minutes each were variable. That is, in one experiment the hemorrhage was increased by 90 per cent in $\frac{1}{2}$ minute after irrigation for 5 minutes and the effect lasted for 5 minutes, then returned promptly to the previous level. In the other experiment, the bleeding was diminished by 60 per cent in 11 minutes and the effect lasted for 16 minutes, then the bleeding returned to the previous level.

Under the conditions, thromboplastin appears to be uncertain and probably entirely inactive as a local hemostatic. The effects of such factors as pressure and foreign bodies (gauze, cotton-fibre, etc.) in the application of thromboplastin to bleeding wounds should be borne in mind, as with all other agents, before

⁵ A portion of the material was furnished by Squibb and Company.

accepting final judgment as to the activity and efficiency of the product per se.

Serums. Beef serum; two applications with and without citrate for 5 minutes each increased the bleeding by 66 and 45 per cent in 1 and 2 minutes and the effects lasted for 8 and 6 minutes, respectively. Horse serum: Five minute applications were made. In one experiment the fresh serum without citrate reduced bleeding by 44 per cent in 11 minutes and the effect lasted for 22 minutes, which is less effective than the average stoppage (10 to 15 minutes) of untreated wounds. In another experiment (with citrate) the bleeding remained unchanged for 16 minutes. In the majority of experiments with old serum bleeding was increased, and this was also the case with fresh serum preserved with 0.2 per cent trikresol. That is, results with old serum with and without citrate ranged from a diminution by 60 per cent to an increase by 125 per cent in hemorrhage in about 1 minute after the application and the effects lasted approximately 20 minutes in each case. Serum preserved with trikresol increased bleeding by 70 and 120 per cent in 1 minute (each case) and the effects lasted for 11 and 16 minutes, respectively.

It may be concluded that the group of thromboplastic substances, which are said to aid the normal clotting of blood, including kephalin, coagulen, thromboplastin and horse and beef serums, did not beneficially affect local hemorrhage when applied directly to wounds of the dog's foot pad by irrigation with and without 1 per cent citrate. Systemic (intravenous) administration of these agents, which will be discussed in another paper, also did not produce hemostasis, independent of changes in blood pressure. Indeed, in a recent monograph on this class of substances by Hugh Maclean (18 a) it is doubted if the lipins (Kephalin, etc.) constitute the thromboplastic substance of the tissues.

Miscellaneous agents

Calcium. Three irrigations of 5 minutes each with calcium chloride (2 per cent) in citrate and normal saline slightly les-

sened hemorrhage in about 6 minutes (from 9 to 57 per cent) and the effects lasted for 12 minutes.

Quinine. Irrigation with a 0.1 per cent solution for 5 minutes lessened bleeding by 43 per cent in 5 minutes and the effect lasted for 7 minutes.

Hydrastis and constituents. The results of three out of four irrigations with the fluid extract of hydrastis (alcohol free) indicate an increase in bleeding from 31 to 100 per cent in 1 minute and the effects lasted for about 18 minutes in each case. One irrigation lessened bleeding by 55 per cent. Two irrigations with berberin sulphate (saturated solution) for 5 minutes each temporarily (from 6 to 11 minutes) increased bleeding by 10 to 26 per cent. Two irrigations with hydrastinine hydrochloride (0.1 per cent) for 1 and 5 minutes increased bleeding by 44 to 50 per cent and for 22 and 11 minutes, respectively.

Ergot. Three irrigations with the alcohol-free fluid extract for 1 and 5 minutes increased bleeding from 63 to 174 per cent and the effects lasted for 10 to 17 minutes. The effects are somewhat similar to histamin.

Menthol. This would be of interest in connection with pharyngeal and nasal applications. Two irrigations with saturated solutions (at 38°C.) for 5 minutes each produced no effect on local hemorrhage in 15 and 30 minutes, respectively.

Nitrite. Four irrigations for 5 minutes each with 1 and 5 per cent solutions increased bleeding in $\frac{1}{2}$ to 1 minute by 55 to 267 per cent and the effects lasted for 8 to 16 minutes, the effects presumably being due to local vasodilatation. The wounds were brown from methemoglobin formation.

Emetin. This has been used both as a local and systemic (19) hemostatic. Two irrigations for 5 minutes each with saturated (less than 1 per cent) solutions of emetin hydrochloride increased bleeding by 31 to 100 per cent for 1 to 2 minutes and the effects lasted from 10 to 11 minutes. These results do not agree with the local hemostatic claims and uses.

Chloral. Four irrigations with 5 per cent solutions of chloral for 5 minutes each increased bleeding from 3 wounds in $\frac{1}{2}$ to 1 minute by 25 to 200 per cent and the effects lasted for 5 to 11

minutes. The bleeding from one wound was first increased by 25 per cent for 6 minutes, then lessened by 50 per cent for 13 minutes.

Chlorine products. Chlorazene, dichloramine-T and Dakin's hypochlorite solution were tried. These products are said to increase the flow of blood in wounds irrigated with them. *Chlorazene:* A 1 per cent solution gave variable results with three irrigations. After applications for 5 minutes in each case hemorrhage was decreased by 70 per cent in 11 minutes from one wound and the effects lasted for 11+ minutes. Bleeding from the other two wounds was increased by 250+ and 233 per cent in $\frac{1}{2}$ minute and the effects lasted for 3 and 15 minutes. The bleeding then returned to the previous level. One irrigation with a 2 per cent solution increased bleeding by 200 per cent in $\frac{1}{2}$ minute and this lasted for 22 minutes. *Dichloramine-T:* This is used as a solution in oil in which a higher concentration is obtained. Saturated solutions of the product in 1 per cent citrate in three irrigations for 5 minutes each increased bleeding by 30 to 253 per cent in $\frac{1}{2}$ to 1 minute and the effects lasted for 6 to 15 minutes. The bleeding in both cases returned to the previous level.

*Dakin's hypochlorite solution.*⁶ Two irrigations for 5 minutes each were made. Bleeding was increased by 80 and 540 per cent almost immediately on application and the maximal increases were demonstrated in both cases in 1 minute after the end of application and the effects lasted for 15 and 12 minutes, respectively. The presence of citrate in the hypochlorite in one irrigation caused a smaller (80 per cent) increase in bleeding, the other irrigation (giving an increase by 54 per cent) was made without citrate.

The effect of the citrate is presumably due to hypertonicity and alkalinity, both of which tend to lessen bleeding as indicated by experiments in the fore-part of the paper. Conversely the absence of alkalinity, also slight acidity, tends to increase bleed-

⁶ The following formula was used: Sodium carbonate (dry) 35 grams; distilled water 2500 grams; dissolve and add calcium chloride (C.P.) 50 grams. This was allowed to stand 30 minutes, siphoned and filtered through cotton; then 10 grams boric acid were added.

ing. From this it is more than probable that the increase in bleeding produced by Dakin's hypochlorite solutions is not due to the alkalinity (13).

With the object in view of adding an efficient, yet harmless, hemostatic to Dakin's solution to reduce the marked increase in bleeding produced by it, the following rough trial with epinephrin was made. A wound was irrigated with Dakin's solution and a marked increase in bleeding was observed. The wound was next irrigated with Dakin's solution containing 1:10,000 epinephrin, and again later with the solution containing 1:1000 epinephrin, but in both trials an increase in bleeding was noted as with the hypochlorite alone. The wound was now irrigated with 1:1000 epinephrin alone and the bleeding ceased almost immediately, and the arrest lasted for about 30 minutes. It was noted that when epinephrin was added to Dakin's solution, the solution developed a pink color, reaching a deep violet within a minute or so, apparently destruction of the epinephrin as by other oxidizing agents, and on standing. This probably accounts for the unsuccessful attempts to reduce the bleeding.

Salicylate. Salicylic acid is sometimes used as a local antiseptic dusting powder for wounds, the effects being due to the salicyl radicle. A 1 per cent solution with which a wound was irrigated for 5 minutes increased bleeding moderately (by 15 per cent) in 3 minutes and this lasted for 8 minutes and then returned to the previous level.

Gelatin. Gelatin has been advocated both as a systemic and local hemostatic. When the effects were favorable this has been attributed to the presence of calcium. The results obtained in my experiments were variable. Two irrigations with 5 per cent solutions at 38°C. for 5 minutes each were made. One lessened bleeding by 40 and another increased it by 76 per cent in 10 and 6 minutes and the effects lasted for 15 and 13 minutes, respectively. The bleeding then returned to the previous level in both experiments.

Peptone. Peptone has been advocated as a hemostatic by Nolf (20). The results with 5 and 10 per cent solutions were variable. Two irrigations for 5 minutes each with a 5 per cent solution increased bleeding by 9 and 38 per cent in 6 minutes

and the effects lasted for 10 and 11 minutes, respectively. Two irrigations with a 10 per cent solution for 5 minutes each lessened bleeding by 52 and 54 per cent in 6 and 16 minutes and the effects lasted for 17 and 21 minutes, respectively. The difference may be due to greater hypertonicity, i.e., greater dehydration of the wound. On the whole the results are variable and not striking.

Turpentine. This has been reported (21) as a hemostatic. It could not be used with citrate. An irrigation with the whole oil for 5 minutes reduced the bleeding by 33 per cent in $\frac{1}{2}$ minute and the effect lasted for 5 minutes. Left to itself without irrigation, and providing there is no fall in blood pressure, bleeding from a foot-pad wound gradually diminishes and stops completely in about 10 to 15 minutes, when the wound becomes covered with a clot, but there are great variations. By comparison with such a control it appears that oil of turpentine scarcely contributes to the stoppage of bleeding, although this could not be definitely settled by the method used.

Camphor. The results from two irrigations for 5 minutes each were variable. In one the bleeding was increased by 50 per cent in 1 minute and the effect lasted for 15 minutes. In the other bleeding was decreased by 75 per cent in 16 minutes and the effect lasted for more than 16 minutes. These results sustain the general reputation enjoyed by camphor in its effects on other functions, namely, its extreme variability. Dilatation of peripheral vessels by low concentrations of camphor was observed by Likhatcheva (22a).

Veratrum and aconite. Irrigations with the alcohol-free tinctures produced no changes in bleeding in 20 minutes in each case.

Atropine. A 0.1 per cent solution was used and irrigations were made for 5 minutes each. In one experiment bleeding was increased by 117 per cent in 11 minutes and the effect lasted for 16 minutes. In another experiment bleeding was diminished by 50 per cent in 2 minutes and the effect lasted for 7 minutes. According to Berezin (23) atropin dilates cerebral vessels, but not vessels of other regions. My results are too variable for drawing definite conclusions.

Permanganate. Hemostatic action has been claimed for this salt (22). Two five minute irrigations were made with 1% and 2% solutions. Bleeding was increased by 11% in one experiment and diminished by 11% in another.

Comparative value of some local hemostatics

Certain of the agents, either desirable or those already in use and those which were effective in diminishing and arresting bleeding, were compared and their efficiency estimated. This was done by taking in consideration the various factors tabulated in table 1, assigning to epinephrin the value of 1. The average numerical values for each of the drugs compared is about as follows: Epinephrin 1, pituitary extract $\frac{1}{4}$, tyramin $\frac{1}{14}$, acetic acid $\frac{1}{250}$, ferric chloride $\frac{1}{600}$, quinine-urea hydrochloride $\frac{1}{625}$, tannin $\frac{1}{5000}$, sodium bicarbonate $\frac{1}{25,000}$, barium chloride $\frac{1}{50,000}$, cane-sugar $\frac{1}{84,000}$, sodium chloride $\frac{1}{500,000}$.

From this it is seen that epinephrin was the most efficient of all the agents tested. As pointed out in the forepart of the paper its action is fleeting and the bleeding later is increased. The effects are, in general, directly proportional to the concentration employed and the duration of application. The curve in Figure 2 illustrates this. The remaining agents are distinctly inferior. Of the astringents ferric chloride is superior to tannin. Pituitary is close to epinephrin, tyramin next and then acetic acid. Of all these agents epinephrin, pituitary extract, tyramin and dilute acetic acid are least objectionable. Bicarbonate appears to be more efficient than sodium chloride. Barium chloride (10 per cent) is also quite effective, but until more is known about its possible systemic toxicity arising from its application to wounds its use had better be curtailed. The remaining agents are so weak that no further consideration of them is necessary. The objections to the use of ferric chloride are well known.

DISCUSSION

In general it may be stated that the local application of vasoconstrictor and astringent agents diminishes or arrests local hemorrhage, while vasodilator and irritating agents (without astringent

action) increase local bleeding. There is some uncertainty regarding the various thromboplastic agents such as kephalin, coagulen and thromboplastin, because of the experimental conditions, although kephalin exerts its ordinary thromboplastic activity in the presence of oxalate and this is perhaps even increased in citrated blood (concentration of citrate unknown).⁷ The treatment of the wounds with kephalin was one to ten times as long as is ordinarily required to produce clotting of the blood in vitro and the concentrations were also greater yet the bleeding continued unchanged or was actually increased.

The effects of the various local anesthetics tested on local hemorrhage generally agree with their vascular effects. The use of orthoform in gastric ulcer and as a dusting agent on wounds should be guarded, since it tends to increase bleeding.

The following agents for which favorable claims of hemostatic action have been made are discredited by the results of this investigation; stypticin (cotarnine hydrochloride), styptol (cotarnine phthalate), antipyrin, peptone and emetin. Local bleeding is increased rather than lessened or arrested by these agents.

The most efficient and desirable hemostatic of all the agents that were tested is epinephrin. However, its action is temporary and almost invariably the bleeding is increased as an after effect, and therefore, it should not be relied upon for effecting permanent hemostasis. Tyramin and pituitary extract act similarly though not quite as effectively, and they do not increase the bleeding later.

The astringents as a class were effective, though variably, in diminishing bleeding. The most effective were ferric chloride and tannin. Alum was disappointing. With high concentrations the bleeding was even increased.

The remaining agents, for various reasons, would be unsuitable hemostatics and merit no further discussion. It is not known what effect these would have on the coagulation time of blood, but it may be assumed that this would not be favorably affected by the majority tested.

Considering the numerous factors involved in their practical

⁷ Private communication from Professor Howell.

application, the results of this investigation and a study of the literature leave considerable to be desired as to the real merits of the general class of local hemostatic agents. The situation is aptly summarized by Hawkins (24), who, in 1832, having investigated a number of styptics, concluded:

If then pressure for a few minutes upon a wounded artery can permanently prevent hemorrhage, when that pressure is made with compresses dipped in various fluids, which neither produce contraction of artery nor facilitate coagulation, we are justified in concluding that if styptics are employed the cessation of the hemorrhage is to be ascribed principally, if not entirely, to the pressure or at all events in a minor degree to the action of the styptic.

The recognized importance of other factors and procedures (25) leaves the importance of hemostatic agents in doubt.

Indeed as far back as 1657 Rhaenodaeus (26) advised

That thou mayst stay bleeding, touch the part with thy ring finger, and repeat these words, Socnon, Socnon, twenty-seven times, or as oft as thou wilt, till the issue cease; it is good, and much profitable, if you rightly consider it.

CONCLUSIONS

1. Beginning with the most efficient, the order of efficiency of the more important of all the hemostatic agents tested is epinephrin, pituitary extract, tyramin, acetic acid, ferric chloride, quinine-urea-hydrochloride, tannin, sodium bicarbonate, barium chloride, cane-sugar, sodium chloride. A number of other agents, which were tried, can lessen local hemorrhage in variable degrees, but on the whole they are inferior and undesirable for various reasons.

2. The following among the more important of this class, and for which hemostatic claims have been made, were found to increase bleeding on local application; cotarnine salts (stypticin and styptol), antipyrin, peptone, emetin, sometimes alum. Orthoform (1 per cent solution) also quite markedly increased local bleeding.

3. Under the conditions, kephalin, coagulen and thromboplastin were all variable, or did not affect the course of bleeding.

REFERENCES

- (1) HANZLIK: J. Pharm. Exp. Therap., 1918, x, 523.
- (2) LISIN: Arch. intern. pharmacodyn., 1907, xvii, 465.
- (3) FREY: Zeit. exp. Path. Therap., 1910, vii, 8.
- (4) PALMER: Proc. Soc. Exp. Biol. Med., 1917, xiv, 175.
- (5) HARTMANN AND FRASER: Am. J. Physiol., 1917, xlv, 353.
HARTMANN AND KILBORN: Am. J. Physiol., 1918, xlv, 111.
- (6) GRUBER: Am. J. Physiol., 1918, xlv, 302.
- (7) HEWLETT: Arch. Int. Med., 1918, xxi, 411.
- (8) SOLLMANN: J. Pharm. Exp. Therap., 1918, xi, 69.
- (9) BASTEDO: Materia Medica, Pharmacology and Therapeutics, 2d ed., 1918.
- (10) MORRIHY: Arch. farm. sper., 1917, xxiv, 129.
- (11) ADLER, I.: J. Pharm. Exp. Therap., 1916, viii, 297.
- (12) TAYLOR: Practitioner, London, 1917, xcvi, 413.
- (13) GITHENS AND MELTZER: J. Am. Med. Assoc., 1918, lxx, 1115.
- (14) MACHT: J. Pharm. Exp. Therap., 1918, xi, 389.
- (15) HOWELL: Am. J. Physiol., 1912, xiii, 1.
McLEAN: Am. J. Physiol., 1916, xli, 250.
McLEAN: Ibid., 1917, xliii, 586.
TORRACA: Lo sperimentale, 1915, lxix, 493.
HESS: Proc. Soc. Exp. Biol. Med., 1915, xii, 117.
BATTELLI: Compt. rend. soc. biol., 1910, lxviii, 789.
- (16) HIRSCHFELDER: Lancet, 1915, ii, 542; Berl. Klin. Woch., 1915, lii, 976.
- (17) CECIL: J. Am. Med. Assoc., 1917, lxviii, 628.
- (18) HURWITZ AND LUCAS: Arch. Int. Med., 1916, xvii, 543.
- (18a) MACLEAN, HUGH: Lecithin and allied substances. The Lipins, 1918, p. 179, Longmans, Green & Co.
- (19) FLANDIN: Presse médicale, 1913, September, 24, p. 777.
- (20) NOLF: Bull. acad. méd. Belg., 1913, xxvi, 485; Id: Ibid, 1916, lxxix, 689.
- (21) ANON: Year book Am. Pharm. Assoc., 1915, p. 257.
- (22) ANON: Lekařske Rozhledy, 1910, xviii, 30.
- (22a) LIKHATCHEVA: Russkii Vrach, 1916, xv, 483.
- (23) BEREZIN: Russkii Vrach, 1916, xv, 513.
- (24) HAWKINS: Med. Chir. Trans., London, 1832, xvii, 121.
- (25) DEAN, L. W.: The Laryngoscope, May, 1916.
- (26) RHAENODAEUS: Dispensatory, Tomlinson's translation, 1657, Book I, p. 22.

THE EFFECTS OF VARIOUS SYSTEMIC AGENTS ON SUPERFICIAL HEMORRHAGE

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In order to make the studies on hemostatics more complete, a number of the more important local agents were studied when administered systemically. Some of these, such as tyramin and pituitary extract, have not been previously investigated from this standpoint, and might be promising. Others, such as the thromboplastic agents, have either not been investigated, or the lack of sufficient controls and consideration of other factors, which might be responsible for the alleged beneficial effects, require further and more careful study. Much of the evidence consists of unsubstantiated clinical opinion. Furthermore, in view of the uncertain and negative results obtained with these agents when applied locally, as indicated in a previous paper (1), it seemed worth while to test them also systemically, as otherwise it might be considered doubtful if all conditions favorable to their action had been met. This could be fulfilled by direct injection of the material into the circulation without citrate and observing the effect on bleeding from the denervated dog's foot pad, taking into consideration the changes in the blood pressure.

The methods used were essentially the same as described in a previous publication (1). Dogs were used, and, as a rule, the drugs were injected intravenously; in some instances, intramuscularly and subcutaneously. This will be indicated in the text.

Any changes in local bleeding produced by the method of study used must be through local effects on the blood vessels, exclusive of the central nervous system, or on the blood itself

(such as viscosity, etc.), providing the blood pressure remains unchanged. Changes in pulse rate or volume, unless profound, or in respiration, are practically without influence. Slowing of the pulse would tend to lessen the bleeding. An increase in pulse rate would tend to have the opposite effect. However, it was observed that when the pulse rate was increased by small doses of epinephrin in the beginning of its action, the bleeding was instantly or almost immediately diminished or stopped. It is clear from this that a mere increase in pulse rate is not sufficient to augment bleeding. A further consideration of these factors is unnecessary, since no profound or important changes in pulse or respiratory rate were observed.

The most important external factor concerned in changes in local bleeding is the systemic blood pressure. This was carefully observed in all experiments, recording the pressure directly from the carotid by means of a damped mercury manometer in the usual way.

Unless vasoconstriction occurs, even a slight increase in blood pressure is almost invariably accompanied by an increase in bleeding. It is obvious, therefore, that changes in blood pressure may not be overlooked as the causative factors, if changes in bleeding and blood pressure correspond. On the other hand, if no changes occur in both the course of hemorrhage and the blood pressure it cannot be said that the action of the agent would be the same under normal or other conditions. The action might be on the blood or other functions. It will be presently indicated that a comparison on this basis is valuable in forming an estimate of the hemostatic properties of the different agents tested.

For external reasons it was necessary to bring this work to a close before completion, but the publication of the results thus far obtained was thought desirable. These are presented in table 1 and classified in table 2. An illustration of the course of experiment 2 and results with the more important agents are shown in the accompanying figure.

TABLE 1*

The effects of various agents (administered systemically) on bleeding from the dog's foot-pad

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	DOSAGE INTRAVENOUSLY PER KILOGRAM	MAXIMAL CHANGE IN BLOOD PRESSURE	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLOOD PRESSURE	DURATION OF CHANGE IN BLOOD PRESSURE	MAXIMAL CHANGE IN BLEEDING	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLEEDING	DURATION OF CHANGE IN BLEEDING	PREVIOUS LEVEL OF BLOOD PRESSURE
Epinephrin									
	kgm.		per cent	min-utes	min-utes	per cent	min-utes	min-utes	mm.
45	8.0	0.1 cc. of 1:1000	+816.0	4	20	-100.0	10	20	12
46	5.0	0.1 cc. of 1:1000	+125.0	3	46	-75.0	5	40+	40
50	7.0	0.5 cc. of 1:1000	+257.0	2½	5	-100.0	6	13	35
51	9.1	0.5 cc. of 1:1000	+186.0	2	8+	-33.0	8	8+	35
52	10.5	0.5 cc. of 1:1000	+267.0	5	15	-30.0	10	15	15
62	10.5	0.25 cc. of 1:10,000	+55.0	1½	3	-80.0	3	6	100
62	10.5	0.125 cc. of 1:10,000	+50.0	2½	7	-80.0	3	9	83
Pituitary extract									
51	9.1	0.1 cc.	+133.0	5	20+	+23.0	5	12	30
52	10.5	0.5 cc.	+157.0	2	20	+180.0	2	20	15
62	10.5	0.1 cc.	+26.0	3	17	+31.0	5½	10	100
62	10.5	0.5 cc.	+26.0	20	25+	-22.0	8	10	90
Tyramin									
51	9.1	0.002 gram	+38.0	2	7	+25.0	2	12	45
62	10.5	0.002 gram	+30.0	1½	7	-76.0	1½	11	100
63	9.2	0.002 gram	+25.0	1½	9	-20.0	1½	12	80
Coagulen (Ciba)									
50	7.0	0.05 gram	+43.0	2	5	+71.0	2	13	35
50	7.0	0.1 gram	+37.0	1	5	+27.0	2	5	35
50	7.0	0.1 gram	-33.0	8	15+	-20.0	3	8	45
61	10.5	0.5 cc. of 5 per cent	-25.0	9	20+	-30.0	9	9+	40
62	10.5	1.0 cc. of 5 per cent	-5.6	1½	5	-22.0	6	10	90
Normal saline (0.9 per cent NaCl)									
46	5.0	10.0 cc.	+233.0	20	22	+67.0	15	20	20
Sodium nitrite									
46	5.0	0.004 gram	-69.0	8	8+	+100.0	5	8+	70

TABLE 1—Continued

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	DOSAGE INTRAVENOUSLY PER KILOGRAM	MAXIMAL CHANGE IN BLOOD PRESSURE	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLOOD PRESSURE	DURATION OF CHANGE IN BLOOD PRESSURE	MAXIMAL CHANGE IN BLEEDING	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLEEDING	DURATION OF CHANGE IN BLEEDING	PREVIOUS LEVEL OF BLOOD PRESSURE
Tincture of digitalis									
46	kgm. 5.0	2.0 cc.	+ 36.0	12	12+	-100.0	10		22
Fluidextract hydrastis									
48	5.5	0.02 cc.	- 56.0	10	10+	+140.0	5	10	45
Fluidextract ergot									
48	5.5	0.3 cc.	+ 40.0	5	8	- 38.0	10	10+	18
Beef serum									
58	12.5	1.0 cc.	None			None		in 7	28
58	12.5	25.8 cc.	- 30.0	10	10+	+ 38.0	5	10+	20
Horse serum									
59	9.0	1.0 cc.	- 14.0	3	20+	- 20.0	3	10+	35
59		2.0 cc.	- 11.0	8+	8+	+ 3.3	3	10+	32
62	10.5	1.0 cc.	+ 4.3	1½	14	+ 40.0	2	10	115
62		0.5 cc. {First	+ 4.0	5	7½	- 16.0	6	6	120
		Later	- 4.0	11	14	- 13.0	10	13	120
Cotarnine hydrochloride (stypticin)									
62	10.5	0.005 gram	- 35.0	1½	4	- 46.0	4	6	98†
63	9.2	0.005 gram	- 30.0	1	12	-100.0	1	3	86
Kephalin‡ (Howell)									
62	10.5	1.0 cc. of 5 {First	+ 5.6	1½	5	- 27.0	1½	5	90
		per cent {Later	- 5.6	5	4	+ 6.7	6	2	95
		1.0 cc. of 5 per cent	+ 14.0	2½	20+	+ 62.0	2½	20+	73
63	9.2	1.0 cc. of 5 per cent	12.5	15	25	+250.0	11	22	80
Thromboplastin (Squibb)									
62	10.5	0.5 cc.	- 40.0	17	17+	- 80.0	2	17+	120
62	10.5	0.5 cc.	-100.0	15	§	-100.0	15	§	90
63	9.2	1.0 cc. intramuscularly	Unchanged	for 39 minutes		Unchanged	for 39 minutes		80

TABLE 1—*Concluded*

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL	DOSAGE INTRAVENOUSLY PER KILOGRAM	MAXIMAL CHANGE IN BLOOD PRESSURE	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLOOD PRESSURE	DURATION OF CHANGE IN BLOOD PRESSURE	MAXIMAL CHANGE IN BLEEDING	TIME OF APPEARANCE OF MAXIMAL CHANGE IN BLEEDING	DURATION OF CHANGE IN BLEEDING	PREVIOUS LEVEL OF BLOOD PRESSURE
Gelatin									
	kgm.		per cent	min-utes	min-utes	per cent	min-utes	min-utes	mm.
63	9.2	2.0 cc. of 5 per cent intravenously	+ 12.5	5	17+	+131.0	5	15+	80
Peptone									
63	9.2	1.0 cc. of 5 per cent subcutaneously	Unchanged for 30 minutes		Unchanged for 30 minutes		85		
Emetin hydrochloride									
63	9.2	0.001 gram subcutaneously	Unchanged for 15 minutes (at first)		Unchanged first 15 minutes		86		
			6.1 per cent 15 min-utes later	55	- 70.0	30			

* The plus (+) sign means increased; minus (—) sign, diminished.

† Later bleeding was increased by 21 per cent in three minutes without change in blood pressure.

‡ Fresh; prepared by Howell's method.

§ Permanent.

TABLE 2

Classification of various agents acting systemically on hemorrhage from the dog's foot-pad*

BLEEDING DIMINISHED	BLEEDING INCREASED	BLEEDING VARIABLE OR UNCHANGED
Cotarnine hydrochloride (stypticin)	Asphyxia	Beef serum
Digitalis	Gelatin	Coagulen (Ciba)
Epinephrin	Kephalin (Howell)	Emetin (subcutaneously)
Ergot	Nitrite	Horse serum
Hydrastis	Normal saline	Pituitary extract
Thromboplastin (Squibb)		Thromboplastin (Squibb) (intramuscularly)
Tyramin		

* Irrespective of changes in blood pressure. The relation of this to changes in bleeding is considered in the text, hemostasis by some agents being accomplished at the expense of a considerable fall of blood pressure as with cotarnine, for instance.

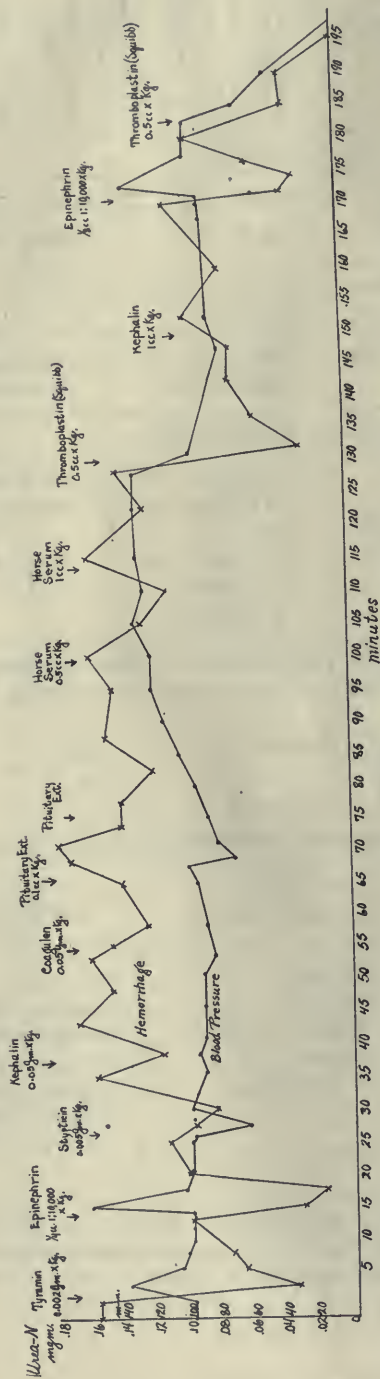


FIG. 1. EXPERIMENT 2. THE EFFECTS OF VARIOUS AGENTS SYSTEMICALLY (BY INTRAVENOUS ADMINISTRATION) ON BLOOD PRESSURE, AND HEMORRHAGE FROM THE DOG'S FOOT-PAD

Fresh kephalin, prepared by Howell's method from pigs' brains, was used; horse-serum was fresh and preserved with 0.2 per cent trikresol.

I. EPINEPHRIN AND RELATED AGENTS

Epinephrin. The data obtained indicate that epinephrin was the most effective of all the agents tested. The changes in bleeding and blood pressure are reciprocal, and practically irrespective of the original level of pressure. That is, the rise of blood pressure produced by epinephrin was always accompanied by a prompt and marked diminution or arrest (from 30 to 100 per cent) of hemorrhage. The maximal hemostatic effects appeared in about 8 minutes and the hemostasis lasted for about 15 minutes (range from 8 to 40 minutes). There was a relative rise of blood pressure by 125 to 816 per cent and this was dependent upon dosage, which was variable (0.5 to 1 cc. per kilogram of 1:10,000).

Tyramin. In the majority of three experiments with tyramin, bleeding was diminished, but not near as markedly (20 to 76 per cent) as with epinephrin, and the dosage was much greater. Hence, it appears to be less effective.

Pituitary extract. Results with pituitary were variable. In two experiments, 51 and 52, there was an increase in bleeding with increase in blood pressure. In experiment 62 a small dose produced an increase in bleeding with a fall of pressure, and a larger dose (0.5 cc. per kilogram) in the same animal reduced the bleeding while the blood pressure was raised. From all this pituitary does not appear to be reliable as a hemostatic.

II. THROMBOPLASTIC AGENTS, INCLUDING SERUMS

Under the condition of wound irrigation, systemic administration of the thromboplastic agents (kephalin, etc.) can not throw any more light on the efficacy of their natural rôle¹ in the stoppage of bleeding than local application. However, it is possible to compare their effects with some other effective hemostatic agents, such as epinephrin and tyramin, taking in consideration the changes in blood pressure. The following were tested:

¹ In a recent monograph Hugh Maclean (Lecithin and Allied Substances, The Lipins, 1918, p. 179—Longmans, Green & Co.) doubts if the lipins (kephalin, etc.) constitute the thromboplastic substance of the tissues.

kephalin (Howell), thromboplastin (Squibb), and coagulen (Ciba). Experiments with horse and beef serums are also included.

The results indicate that thromboplastin, coagulen, and kephalin were variable or ineffective as hemostatics when injected intravenously. The course of the bleeding was parallel with the changes in the blood pressure. That is, bleeding generally increased with increase in blood pressure and vice versa. The effects were, therefore, chiefly mechanical.

In experiment 63, thromboplastin, in higher dosage than recommended by mouth, was injected intravenously with no changes in bleeding and blood pressure for 39 minutes.

The intravenous injection of 2 per cent kephalin is claimed by Hess (2) to shorten the coagulation time of blood. Although regarded as valuable, though temporary, the intravenous use of these agents may be dangerous according to Lee and Vincent (3). The results of my experiments do not support the favorable claims that have been made.

It is possible that the effects would be different with prolonged administration, and it was fully intended to carry this out, but for unavoidable circumstances the experiments fall short of this.

III. COTARNINE HYDROCHLORIDE (STYPTICIN)

In two experiments on two different animals intravenous injections of 0.005 gram per kilogram of cotarnine hydrochloride reduced bleeding in both and the blood pressure fell also, as would be expected. The effects are attributed to the changes in blood pressure, but whether the mechanism involves the vessels or the heart is not known, though presumably this consists chiefly of vascular dilatation. A prompt recovery of the blood pressure and a return of bleeding to the previous level occurred in both experiments. Cotarnine, therefore, does not seem to act as a systemic hemostatic per se without a marked fall of blood pressure.

IV. MISCELLANEOUS AGENTS

One experiment each was performed with the agents described in this section.

Digitalis. A large dose (2 cc. per kilogram) of the tincture arrested bleeding while the blood pressure rose, presumably due to vaso-constriction.

Ergot. A fatal dose of the fluid extract lessened bleeding while the blood pressure rose.

Normal saline (0.9 per cent NaCl). 10 cc. per kilogram were found to increase bleeding by 67 per cent and the blood pressure increased by 233 per cent. Apparently this was purely mechanical.

Nitrite. This increased the bleeding while the blood pressure fell, presumably due to vasodilatation.

Gelatin. An injection of 2 cc. per kilogram of a warm 5 per cent gelatin solution increased bleeding by 131 per cent and the blood pressure was increased by 12.5 per cent. It is not known whether the viscosity of the blood was altered, but the total quantity (about 20 cc.) of gelatin injected seems considerable. The usual sustaining effect on the increased blood pressure was observed, and the increase in bleeding seemed to be maintained.

Peptone. A subcutaneous injection of 1 cc. of 5 per cent per kilogram did not affect the course of bleeding and blood pressure for 39 minutes. Peptone has been advised as a hemostatic by Nolf (4).

Emetin. One animal (experiment 63), which had previously received an intramuscular injection of thromboplastin (Squibb) without any effect for 39 minutes, was injected subcutaneously with 0.001 gram per kilogram of emetin hydrochloride. This drug has been advised as a hemostatic (5), presumably because of its blood pressure lowering effects by nausea, etc. However, in this experiment, the bleeding and blood pressure remained unchanged during the first 15 minutes after injection. Later, i.e., at the end of 30 minutes after injection, the blood pressure fell by about 6.1 per cent and bleeding by this time was diminished by about 70 per cent. When this experiment was terminated, autopsy was made and this revealed extensive hyperemia of the entire alimentary canal, particularly in the vascular areas of the greater and lesser curvatures of the stomach. In these areas there were also many petechial hemorrhages. The dimi-

nution in the bleeding towards the end of the experiment may therefore be accounted for by the extensive dilatation in the splanchnic area.

Hydrastis. An injection of a therapeutic dose of the fluid extract increased the bleeding while the blood pressure fell. Apparently this was due to peripheral vascular dilatation although the cardiac element was not excluded.

V. CONCLUSIONS

1. The most effective hemostatic agent on superficial bleeding by systemic (intravenous) administration was epinephrin; tyramin somewhat less; pituitary extract was variable. Fatal doses of ergot and digitalis (one experiment each) also lessened and arrested, respectively, the bleeding.

2. The effects of the following (systemically) on bleeding are roughly parallel to the changes in blood pressure: coagulen (Ciba), kephalin (Howell), thromboplastin (Squibb), horse serum, stypticin, gelatin, saline, emetin and possibly peptone. Nitrite and hydrastis increased bleeding with a fall in pressure. The results with the thromboplastic agents might be different with prolonged administration.

REFERENCES

- (1) HANZLIK: Jour. Pharm. Exp. Therap., 1918, xii, 71.
- (2) HESS: Proc. Soc. Exp. Biol. Med. 1915, xii, 17.
- (3) LEE AND VINCENT: Clev. Med. Jour., 1917, xvi, 65.
- (4) NOLF: Bull. Acad. Méd. Belg., 1913, xxvi, 485; id.: ibid., 1916, xxix, 648.
- (5) FLANDIN: Presse médicale, 1913, September 24, p. 777.

ANTHELMINTICS: THEIR EFFICIENCY AS TESTED ON EARTHWORMS¹

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INTRODUCTION

The war has created a shortage in the supply of the usual anthelmintics, which makes it advisable to utilize the available drugs to the best advantage. Trustworthy and convenient tests of efficiency will therefore be especially useful. Several methods of bio-assay have indeed been employed:

1. The direct therapeutic test on man is not always feasible; it is not altogether reliable, because conditions are not easily controlled in human patients; it therefore requires a large statistical material.

2. The therapeutic test on mammals was employed, for instance, by M. C. Hall and Foster (1). This also suffers, though to a less degree, from the difficulty of exact control of conditions, and therefore requires a larger number of animals.

3. Experiments on the parasites, removed from human or animal subjects and kept alive outside the body under appropriate conditions. The supply of parasites is uncertain, and the maintenance of "normal" conditions is difficult; so that this method has been used mainly qualitatively, especially in ascaris, as in the work of Schroeder (2), Straub (3) and Bruening (4).

4. Earthworms resemble intestinal "worms" in their reactions toward anthelmintics, and have been used qualitatively for investigating the nature of the action of anthelmintics, for instance by Straub (3) and by Trendelenburg (5). Yagi (6) pro-

¹This investigation was partly supported by a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

posed the liquefaction on injection of malefern as a specific quantitative test for this drug.

This general similarity of the reactions of earthworms suggested the hope that simple toxicity experiments would furnish valuable data, with certain limitations. This hope has been realized by the results of the following investigations. They show that all anthelmintics are toxic to earthworms. A substance that is not toxic to these worms is therefore scarcely worth further trial; and a substance that is toxic to earthworms is worthy of consideration as an anthelmintic.

It will of course require very extensive investigation before its value as a clinical anthelmintic is established, for that involves also other considerations—such as the specific efficiency for human parasites in the intestinal tract; and the absence of excessive toxicity or irritation to the patient.

It is not possible, therefore to deduce the clinical availability or even the clinical efficiency from the order of toxicity to earthworms, except in the sense that drugs that are not toxic to earthworms in fairly high dilutions are probably not anthelmintics whilst those that are effective on earthworms will likely be useful clinically, provided that the conditions of absorption, irritation and toxicity are favorable. This is illustrated by the confirmation of the efficiency of the time-honored pumpkin-seed treatment.

There is a case, however, in which the toxicity to earthworms, and the clinical efficiency should be practically parallel; viz., when comparing closely related drugs; and still more, when comparing different samples of the same drug. For this purpose, the earthworm test is theoretically just as good as the therapeutic test on man or other mammals, and it is of course vastly preferable from a practical standpoint. The importance of this is illustrated in the results on different aspidium and pelletierin preparations.

Finally, the test is ideal from the standpoint of simplicity of technic, and availability of material. Practically unlimited numbers of earthworms of suitable size (1 to 4 inches) may be obtained from a leaf-compost heap, even in midwinter. The factor of seasonable variations has not been determined, since nearly all of the experiments were made within a few weeks.

Experiments on earthworms are also very suitable for classroom work. They may be used in place of the difficultly obtainable ascaris to illustrate Straub's spiral experiment on the repulsive effect of santonin—which, incidentally, is not at all characteristic of that drug.

METHODS OF TOXICITY DETERMINATIONS ON EARTHWORMS

The worms (species not determined) are kept in the laboratory in small crocks, partly filled with leaf mold. When experiments are to be made, the crock is inverted, and the worms, which are mostly at the bottom of the mold, are picked up in mass and dropped in a conical "urine glass"¹ filled with tap water. The solutions to be tested are also made with tap-water and placed in urine glasses, 100 cc. in each, and five of the worms are introduced. The size of the worms makes remarkably little differences; but if they differ widely, it is advisable to have several sizes in each glass. When only five worms are put into 100 cc. of water, they keep alive and normally active for more than a week. Much larger quantities, however, become asphyxiated.

In the experimental glasses, the activity of the worms is examined for a few minutes. Irritants generally cause more or less agitation, increased movement, often "whipping." They are again examined after one or two hours, and again on the next day.

Some drugs show peculiarities; thus tannin causes the protrusion of the mouth, strychnin and physostigmin tend to make the worms curl up more or less tightly. When partly depressed, the animals are often club shaped, more marked depression makes them beaded; when dead or nearly so, they become flaccid and soon smeary and diffluent. Little attention, however, was paid to these minor peculiarities.

Notation. The following letters are used for convenience in denoting the conditions of the animals:

F (fatal), if all the worms are dead or nearly so.

S (subfatal), if the worms were merely depressed or if only a part were dead and the others would presumably survive.

¹ In the case of volatile constituents, glass stoppered bottles of 250 cc. capacity were used.

N (normal), if the worms were not visibly affected.

BB (bile salts-bicarbonate) refers to an artificial intestinal juice, viz.: bilein 0.04 per cent; sodium bicarbonate, 1 per cent.

Unless otherwise stated, the description refers to the conditions after 1 day.

The concentrations always refer to per cent.

General results. The general toxicity of the substances tried is shown roughly in table 1. This arranges the compounds, according to their toxicity in five groups. Special attention is called to the following:

1. The high toxicity of mustard oil, explaining the anthelmintic use of the closely related onion and garlic.

2. The high toxicity of copper sulphate, suggests its use in enemas against oxyuris.

3. The anthelmintics that are most efficient clinically also stand high in their toxicity for earthworms, as shown in the second and third columns.

4. Anthelmintics of doubtful clinical efficiency stand low in their toxicity to earthworms, as shown in the fourth column.

5. Substances that are practically non-toxic to earthworms (column 5) are not used as anthelmintics clinically.

Table 2 shows the data in somewhat more detail, and in alphabetical arrangement.

DETAILED DISCUSSION OF THE ANTHELMINTICS

The comparative toxicity is indicated by the class number, the just fatal concentration being: for class I, 0.0001 to 0.0009; for class II, 0.001 to 0.009; for class III, 0.01 to 0.09; for class IV, 0.1 to 0.9; for class V, 1 and over.

The tests were generally made with five worms, as described.

1. ACID, ACETIC (class III). 0.1:100 = F within 1 hour; 0.01:100 = S within 1 day.

0.1:100 produced immediate violent agitation. This cedes promptly to paralysis, the worms being almost dead in three minutes, responding very feebly to pinching. They are found dead in one hour.

TABLE 1
Fatal concentration (parts per 100)

CLASS I	CLASS II	CLASS III	CLASS IV	CLASS V
<p><i>0.0001 to 0.0009</i> Cupric sulphate Mercuric chloride Mustard oil</p>	<p><i>0.001 to 0.009</i> Histamin Naphthalen Oil chenopodium Oleoresin aspidium Paradichlorbenzene Pelletierin tannate Santonin BB Thymol</p>	<p><i>0.01 to 0.09</i> Acid, acetic Acid, hydrochloric Ant. potassium tartrate Areca Cantharidin Cantharis. Granatum (active) Kamala Picrotoxin Piperin Potassium cyanid Pumpkin seed Quinin Saponin Soap Sodium carbonate Squash seed Tannin</p>	<p><i>0.1 to 0.9</i> Aspidium (old) Bilein Capsicum Ferric chlorid Gentian extract Granatum, old Kusso Onion, fresh Pepper, black Quassia, extract Santounin sodium Spigelia</p>	<p><i>1 and over</i> Alcohol (2 x 4) Chalk Glycerin Sodium bicarbonate Sodium chlorid Sodium saccharin</p> <p>CLASS IV AND V</p> <p>Aloes Ant. potassium tartrate</p>
<p><i>1/100 to 1/20 saturation</i> Chenopodium oil</p>	<p><i>1/20 to 1/10 saturation</i> Aspidium oleoresin Thymol Turpentine oil</p>	<p><i>1/10 to 1/5 saturation</i> Betanaphthol Camphor Chloroform Ether</p>		

TABLE II
Limit concentrations tried
(Per 100 cc.)

	OVER FATAL	JUST FATAL	UNDER FATAL
Acid acetic.....	0.1		0.01
Acid hydrochloric.....	0.03		0.01
Alcohol.....	4.0		2.0
Aloe.....			0.1
Ant. potassium tartrate.....			0.1
Areca.....	0.1		0.01
Aspidium rhigome, old.....		1.0	0.33
Aspidium oleoresin solution.....	0.003	0.002	0.001
Beta naphthol.....	$\frac{1}{2}$ sat.		$\frac{1}{25}$ sat.
Bilein.....	0.2	0.1	0.05
Bilein with bicarbonate.....			0.08
Camphor.....	$\frac{1}{2}$ sat.		$\frac{1}{25}$ sat.
Cantharidin.....		0.01	
Cantharis.....	0.1	0.02	
Capsicum.....	0.3		0.1
Chalk.....			sat.
Chenopodium oil.....	$\frac{1}{20}$ sat.		$\frac{1}{100}$ sat.
Chenopodium in solution.....	0.010		0.005
Chloroform.....	$\frac{1}{2}$ sat.	$\frac{1}{20}$ sat.	$\frac{1}{20}$ sat.
Copper sulphate.....	0.001		0.0001
Epinephrin.....			0.0005
Ether.....	$\frac{1}{2}$ sat.		$\frac{1}{20}$ sat.
Ferric chlorid tincture.....	1.0		0.1
Ferric chlorid salt.....	0.08		0.008
Gentian extract.....	1.0		0.2
Glycerin.....			1.0
Granatum, active.....	0.1		0.01
Granatum, old.....	1.0		0.2
Histamin.....		0.001	
Kamala.....	0.1		0.01
Kusso.....	0.25		0.05
Mercuric chlorid.....	0.001		0.0001
Mustard oil.....	0.0025	0.0005	
Naphthalen.....	0.01	0.002	
Onion, fresh.....	1.0	0.2	0.1
Paradichlorbenzene.....	0.01	0.001	
Pelletierin tannate.....	0.005		0.0025
Pelletierin tanret.....	10.0	5.0	1.0
Pepper, black.....	0.125		0.1
Physostigmin.....			0.0006
Picrotoxin.....			0.01
Piperin.....		0.01	

TABLE II—Continued

	OVER FATAL	JUST FATAL	UNDER FATAL
Potassium cyanid.....	0.1	0.03	0.01
Pumpkin seed, peeled.....	1.0	0.1	0.01
Quassia extract.....		0.5	0.05
Quinin hydrobrom.....	0.1		0.02
Saccharin sodium.....			1.0
Saccharose.....			1.0
Santonin (solution).....	0.01		0.001
Santonin sodium.....		0.5	0.2
Saponin.....	0.1		0.01
Soap.....	0.1		0.03
Sodium bicarbonate.....			1.0
Sodium carbonate.....	0.1	0.03	0.01
Sodium chlorid.....			1.0
Spigelia.....	0.5	0.1	0.02
Squash, cold filtrate.....	0.1		0.01
Squash, boiled filtrate.....		2.0	1.0
Strychnin.....			0.01
Tannin.....	0.05		0.01
Thymol.....	$\frac{1}{10}$ sat.		$\frac{1}{30}$ sat.
Thymol.....	0.01	0.002	0.0005
Turpentine.....	$\frac{1}{10}$ sat.		$\frac{1}{30}$ sat.
Tyramin.....			0.02

0.01:100 produced immediate agitation. In an hour, the worms were sluggish. Next day they were normal.

Ch. Darwin (7) cites Perrier that acetic acid is highly toxic to earthworms. Crozier (8) studied the retraction time. He found that the efficiency of a given H-ion concentration increases with the power to penetrate cells.

2. ACID, HYDROCHLORIC (class III). 0.03:100 = F in $1\frac{1}{2}$ hours; 0.01:100 = N for one day.

0.03:100; this experiment was started by placing 1 cc. of 1 per cent HCl in the bottom of a urine glass containing 100 cc. of water. This produced violent repulsive movements. Enough acid was then mixed with the water to make 0.03 per cent. The worms were found dead in 1 hour.

The toxicity of hydrochloric acid is apparently similar to acetic acid. This toxicity of acid must be an important factor in protecting the stomach against the introduction and development

of parasites. *Ascaris* probably reacts more slowly to poisons, because of its resistant skin; but Schroeder (2) found that 0.8 per cent of H_2SO_4 killed *ascaris* in about 2 hours.

3. ALCOHOL (class V). 5:100 = F between $1\frac{1}{2}$ hours and 1 day; 4:100 = F between 5 hours and 1 day; 2:100 = N for 1 day.

5:100; no immediate reaction. Progressive depression after 4 minutes. Almost non-reactive and nearly dead in $1\frac{1}{2}$ hours. Dead on next day.

4:100; in $2\frac{1}{2}$ hours, feeble and flaccid, one alive. Same in 5 hours. Dead on next day.

2:100; practically no change at any time. Slightly depressed after 1 day.

Schroeder (2) found alcohol promptly narcotic to *ascaris*.

4. ALOES (class IV or V). 0.1:100 = N for 1 day.

A filtered watery solution was used. There was practically no effect at any time.

Aloes may therefore be considered useless as an anthelmintic. It is doubtful whether it would have a flushing effect even on oxyuris.

5. ANTIMONY-POTASSIUM TARTRATE (Tartar Emetic) (class IV or V). 0.1 and 0.01:100 = N for 1 day.

0.01:100 had no perceptible effect.

0.1:100 caused slight agitation at first. After a day, the animals were perhaps a trifle sluggish, but firm.

These results do not indicate a promising degree of anthelmintic effect considering that the drug can only be given in small doses. Hall and Foster (1) state that tartar emetic is commonly used against roundworms in horses. Their own tests on pigs did not give conclusive results.

6. ARECA (class III). 0.1 and 0.5:100 = F within 1 day; 0.01:100 = N for 2 days.

This was used as a fresh filtered infusion made from a sample identified by Dr. Henry Kraemer of the University of Michigan, to whom I am indebted for this and for some of the other drugs.

The tests were made in May.

0.5:100; alive, but stiff in 2 hours; dead in 25 hours.

0.1: 100; dead and indifferent in 21 hours.

0.01: 100; no effect for 2 days.

These results indicate that areca would be a fairly efficient anthelmintic. Hall and Foster (1) state that it is frequently prescribed against ascarids in dogs and tapeworms in poultry. Their own experiments on both kinds of animals were not very encouraging.

7. *ASPIDIUM OLEORESIN* (class II). In general 0.002 to 0.003: 100 = F in 1 day; 0.001 to 0.002 = S in 1 day.

Technic. After several preliminary experiments the following procedure was accepted for making the standard tests.

The oleoresin is shaken, and a very small drop (approximately 0.1 cc. is placed on a small tared filter paper, weighed, and rubbed thoroughly with sand in a mortar, adding the BB solution (bilein, 0.04 per cent; sodium bicarbonate, 1 per cent),² using 50 cc. of the solution for 0.01 gm. of the oleoresin. This is filtered, and represents 2 mgm. of the oleoresin per 10 cc. of the solution.

The dilutions are made up in urine glasses as follows:

0.01 gm. per 100 cc. = 50 cc. of the stock solution and water q. s. ad 100 cc.

0.005 gm. per 100 cc. = 25 cc. of the stock solution and water q. s. ad 100 cc.

0.003 gm. per 100 cc. = 15 cc. of the stock solution and water q. s. ad 100 cc.

0.002 gm. per 100 cc. = 10 cc. of the stock solution and water q. s. ad 100 cc.

0.001 gm. per 100 cc. = 5 cc. of the stock solution and water q. s. ad 100 cc.

Five worms are placed in each glass and observed after 24 hours. A standard oleoresin should kill the worms in 0.002 to 0.003 per cent—differing probably a little with the season. Until this point is determined, it may be advisable to check the preparation to be tested against a preparation of known strength (i.e.,

² Probably simple 1 per cent sodium bicarbonate could be substituted; some comparative experiments showed no essential difference.

one that is fatal with 0.003 per cent and only depressant with 0.002 per cent in cold weather).

Influence of season. This was tested on one specimen (III), one determination being made in March, another in May. The results indicate a greater susceptibility in May.

In May as follows:

CONCENTRATION	MARCH	MAY
0.003: 100 0.002: 100	F: practically dead in 2 hours S: in 1 day, one is dead; 4 are curled and depressed, but fairly alive.	F within 18 hours.
0.001: 100 0.0005: 100		{ 1 lot F within 18 hours. 1 lot N within 21 hours. N in 21 hours.

Comparison of specimens of aspidium oleoresin. I was fortunate in securing supplies of the fresh and old oleoresin and other preparations through the kindness of Prof. Ed. Kremers of the University of Wisconsin, and from Parke, Davis & Co. Inasmuch as the Wisconsin material was tested in May, and all the other materials in March, the 2 groups are tabulated separately.

Materials tested in March. The following all killed within a day with 0.003: 100; whilst 0.002 was survived.

Specimen I, an old museum specimen labeled "Benton, Hall & Co., Cleveland," manufactured since 1906.

Specimen II, a recent market specimen, Eli, Lilly & Co. "3696 X 48279."

Specimen III, a recent market specimen, furnished by Parke, Davis & Co., and labeled "2229092."

Specimen IV, an old, returned sample, full of sediment and supposedly inactive, furnished by Parke, Davis & Co.³

With all of the specimens, 0.003: 100 greatly depressed the worms in 2½ hours. They were found dead on the following day.

With 0.002: 100, no effect was noted in an hour.

³ I take pleasure in thanking Parke, Davis & Co. for these specimens.

After a day, the animals were very depressed and clumsy, and just alive. Two worms out of the 20 had actually died.

It is remarkable that the old and presumably spoiled specimens were just as active as the fresh oleoresin. This is also borne out by the Wisconsin series, which, although over 8 years old, measured up fully to the present market samples. Evidently, there need be no fear of the deterioration of the oleoresin. The rhizome itself, however, becomes entirely inactive in time, as will be shown later.

Wisconsin specimens. These comprise some commercial samples, and a series of preparations made by Mr. Netzel and Dr. Du Mez in the year 1909-1910. They may be divided into several groups.

$F = 0.002$; $N = 0.001$. This group includes the following specimens. An ethereal extract prepared by Netzel from green rhizome; and commercial specimens by Eli, Lilly & Co. ("8609 x 5790"); Parke, Davis & Co.; and Squibb ("312760"). The Netzel and Squibb samples had abundant sediment; those of Parke, Davis & Co., and Eli, Lilly & Co. had practically none.

A sample from Stearns & Co. ("63613H") was weak, surviving with 0.005 per cent, but dying with 0.01 per cent. This had no sediment.

Another specimen prepared by Netzel with ether from "good rhizome" containing a sediment, was intermediate. The worms survived 0.003 per cent and died with 0.005 per cent.

An acetone-oleoresin by Netzel approached the Stearns preparation (survived 0.005 per cent, died with 0.0 per cent). The same toxicity was possessed by an *alcoholic extract*.

A benzine extract (Netzel) was fairly active (survived 0.003 per cent, died with 0.005 per cent).

It is again apparent that the oleoresin retains its full activity for at least 8 years. As to solvents, the material was inadequate to express an opinion.

The sediments in aspidium oleoresin. The activity of these was investigated on the old Parke, Davis & Co. specimens, and on one of the Netzel preparations.

Parke, Davis & Co. Specimen IV contained considerable sediment. Microscopically this consisted of rather large spheroids, of a radiated structure, reminding of leucin.

A toxicity comparison was made between the clear supernatant fluid, and the sediment-rich bottom layer, using in each case 0.003:100. The result was the same in both cases: considering depression, but not actual death, in one day.

This shows that the sediment is not greatly more active than the solution; it does not show whether the sediment has any action, since it was contaminated with more or less fluid.

Purified sediment. The sediment was then purified by washing it several times with ether. The liquid and sediment are both soluble in ether, but the sediment more slowly. By quick manipulation it is therefore possible to obtain a white or slightly brownish powder, easily soluble in sodium bicarbonate. This could be separated into a rather coarse sandy portion, of which about 0.015 gram were collected, and a fine powder, formed at least partly by evaporation of the ethereal solution of the coarse powder. About 0.05 gram of this fine powder was gathered. Both sediments were tried in increasing concentrations (bilein-bicarbonate vehicle) until 0.01:100 was reached, without killing the worms. The fine powder produced no visible results. The coarse powder caused depression, but this might be attributable to incomplete removal of the fluid.

Netzel deposit. This also dissolved readily in the bilein-bicarbonate mixture. Its toxicity was just the same as that of the extract from which it separated.

It is safe to conclude that the sediments occurring in some of the oleoresins on standing, are not more actively anthelmintic than the overlying fluid. More probably they have less or no activity, but they were not separated in sufficiently pure form to demonstrate their inactivity.

Effect of boiling aspidium solutions. This promptly destroys or at least lowers the activity. It was tried on two occasions, boiling $\frac{1}{4}$ hour each time; once 0.003 in $\frac{1}{10}$ BB; the other time with 0.005 in $\frac{1}{4}$ BB. In neither case was there any visible effect in one day. In the unboiled solutions, the worms were practically dead in $2\frac{1}{2}$ hours, and quite dead in one day (sample III).

Literature of biologic tests for aspidium. W. Straub (3) observed that *Taenias* survive for a considerable time when kept under suitable conditions in Bunge's solution, but that they die within two hours if 0.01 per cent filicic acid or aspidin had been added. He also found that invertebrates generally are very susceptible to aspidium. This he attributes to direct paralysis of the muscles.

He used an earthworm test similar to the present for determining the relative efficiency of the aspidium constituents. In this connection he found filicic acid to be much more toxic than albaspidin or flavaspidic acid.

Finally he observed that the injection of 0.1 mgm. of filicic acid into a large earthworm killed in 6 to 8 hours, with local liquefaction. (I do not know whether this liquefaction is at all characteristic of aspidium or whether it would not follow any locally toxic agent.) This liquefaction test was later proposed by Yagi (6) as a quantitative method (Fuehner, 9). I do not know whether it has proven successful.

Hall and Foster (1) tried the oleoresin on dogs and cats. They found it highly effective against tenias, doubtful against hookworms, poor against ascarids.

8. OLD DRIED ASPIDIUM RHIZOME (class V). 1:100 = just F in 1 day; 0.33:100 = N for 1 day. The department museum contains a whole specimen, which was there when I took charge, 20 years ago. It is "well-preserved," but has no smell or taste of aspidium. A representative sample was powdered. A 1 per cent cold macerate in bilein bicarbonate, with or without filtration, caused marked depression in an hour. In a day, the worms were at the point of death. A $\frac{1}{3}$ per cent filtrate left the worms quite alive, although somewhat depressed.

The weak anthelmintic action may have been due to tannin. The characteristic efficiency of the fresh drug, as represented in the oleoresin, has evidently been lost with the characteristic flavor, by the drying and age.

9. BETANAPHTHOL (class III?). $\frac{1}{3}$ saturated = F; $\frac{1}{15}$ saturated = N.

This was used only as dilutions of a stock saturated solution, made by triturating the drug with chalk, macerating with water,

and filling and diluting as needed. The same method was also used for aspidium oleoresin, camphor, chenopodium oil, chloroform, ether and thymol. A simple chalk solution had no effect on the worms.

One-fifth saturated betanaphthol produced considerable depression in 15 minutes and 1 hour. Next day, dead and flaccid.

One-twenty-fifth saturated, no definite effect in a day.

Betanaphthol would therefore appear to be an efficient anthelmintic, although inferior to naphthalen or paradichlorbenzene.

10. BILE SALTS⁴ (class IV). 0.2:100 = F; 0.1:100 = S; 0.05:100 = N.

0.2:100 caused agitation on immersion; sluggishness in 1 hour; dead next day.

0.1:100, after 1 day, 2 were dead; 4 alive, but clubshaped.

0.05:100 caused slight agitation on immersion. Normal in 1 day.

Bile salts are therefore distinctly weak anthelmintics. This is, of course, in consonance with the life of the parasites in the intestinal tract.

11. BILE SALTS AND BICARBONATE MIXTURE (class IV). 0.08 bilein and 1 per cent sodium bicarbonate : 100 = S; 0.05 bilein and 1 per cent sodium bicarbonate : 100 = N. 0.08:100 of 1 per cent sodium bicarbonate for one day caused only a doubtful depression. Evidently the bicarbonate does not increase the toxicity of the bile salts. This point was important in connection with the "*BB mixture*," which was used as an artificial intestinal fluid in many of the experiments. This "*BB*" contained 0.04 bilein in 100 of 1 per cent bicarbonate, or about one-third of the fatal concentration. It was generally further diluted after use.

12. CAMPHOR (class III). $\frac{1}{5}$ saturated = F between 18 and 25 hours; $\frac{1}{25}$ saturated = N for 1 day.

Camphor was used as dilutions of a saturated solution, made with the aid of chalk.

The $\frac{1}{5}$ saturated caused considerable agitation on immersion, followed by great depression in 20 minutes. In 1 hour, the worms

⁴ "Bilein-Abbott" was used.

appeared almost dead; after 18 hours, they were in the same condition. In 25 hours, they were dead and beaded.

Camphor thus appears a quite efficient anthelmintic; but efficient doses would probably be dangerous to the patient.

13. CANTHARIDIN (class III). 0.01:100, practically fatal in 1 day.

The cantharidin was dissolved in "bilein-bicarbonate," in which it was apparently soluble.

14. CANTHARIS (class III). 0.1:100 = F in 1 day; 0.02:100 = practically F in 1 day.

0.5 per cent powdered cantharis was infused with bilein-bicarbonate, macerated over night, diluted with water, and filtered.

0.1:100 caused no apparent change in one hour, but death after 1 day.

0.02:100, after one day, 3 were dead, 3 others were almost dead, being of fair tone, but clubshaped.

It is interesting that so specialized an irritant should be so highly toxic to the worms. As a practical anthelmintic, it is of course out of the question.

15. CAPSICUM (class IV). 0.3:100 = F in 1 day; 0.1:100 = N in 1 day.

Capsicum was used as a 0.5 per cent decoction with bilein-bicarbonate, macerated for a day, filtered and diluted.

0.5 per cent (not filtered) was fatal in $2\frac{1}{2}$ hours.

0.3 per cent; normal in one hour; dead and flaccid in 1 day.

0.1 per cent; somewhat depressed in 1 hour; normal for 1 day.

Pungent condiments like capsicum, pepper, mustard, onion, etc., appear fairly toxic to worms; explaining their use as adjuvants in "worm cures." This will be discussed again under "Synergistic Combination."

16. CHALK. Saturated solution = N in 1 day.

This was tried because of the use of chalk in making saturated solutions of different soluble drugs.

17. CHENOPODIUM OIL (class II). 0.010:100 = F in 1 day; $\frac{1}{20}$ saturated = F in 1 day; 0.005:100 = N in 1 day; $\frac{1}{100}$ saturated = N for 1 day.

Chenopodium oil was tried in several ways; namely as saturated solutions after trituration with chalk; and as solutions in bilein, and in bilein-bicarbonate mixture.

Chenopodium oil-chalk trituration. All the dilutions produced considerable agitation on immersion.

Saturated solution: practically dead in 12 minutes; dead in 15 minutes.

$\frac{1}{5}$ saturated solution: dead and beaded in one day.

$\frac{1}{20}$ saturated solution: pale, incoördinated, but lively after 5 hours; dead in one day.

$\frac{1}{100}$ saturated solution; normal in one day.

Chenopodium oil and bilein. A stock solution was made by triturating 0.11 gram of the oil and 0.04 gram of bilein with filter paper, sand and water, to make 100 cc. and filtering.

CHENOPODIUM OIL	BILEIN	
<i>per cent</i>	<i>per cent</i>	
0.11	0.04	Depressed in 10 minutes, dead, flaccid and diffuent next day.
0.011	0.004	Sluggish but fairly normal in 2½ hours. Next day, very weak, clubshaped, but alive.
0.0022	0.0008	Normal for 1 day.

Chenopodium oil in bilein bicarbonate, unfiltered. In this first series the worms were placed in the unfiltered mixture.

CHENOPODIUM OIL	BILEIN	SODIUM BICARBONATE	
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
0.13	0.04	1.0	Depressed in 10 minutes; dead and diffuent on next day.
0.013	0.004	0.1	After 2½ hours, very sluggish, moderate tone. Next day, dead and flaccid.
0.0026	0.0008	0.02	Sluggish and clumsy after 1 day.
0.005	0.04	1.0	Alive in 2½ hours; dead in 1 day.

Filtered chenopodium oil bilein bicarbonate mixture. 0.025 in "BB" immediate marked agitation; after 1 hour very depressed; 2 hours dead; 1 day, dead and flaccid.

0.010 in "BB." Immediate marked agitation; after $3\frac{1}{2}$ hours, very depressed, nearly dead; 1 day, dead and flaccid.

0.005 in "BB." Normal for 1 day.

Bruening (4) found the toxic concentration for ascarids in Ringer's fluid to be 0.01 to 0.02 per cent of the emulsified oil. This therefore requires about twice as much as earthworms. In both cases, the effects consist in temporary stimulation, followed by paralysis in a few hours.

Trendelenburg (5) also described the effects of chenopodium on earthworms as initial stimulation followed by paralysis. He considers this similar to santonin.

Hall and Foster (1) found it a reliable anthelmintic in dogs, causing complete disappearance of ascarids.

The clinical results in man have been highly successful.

18. CHLOROFORM (class III?). $\frac{1}{5}$ saturated = F within 8 minutes; $\frac{1}{20}$ saturated = paralyzed within 6 minutes, but recover in the solution; $\frac{1}{50}$ saturated = N for 1 day.

Chloroform was tried as a saturated solution, made by triturating with chalk, standing, filtering and diluting as needed. All the dilutions depressed at once.

The results indicate that chloroform is an active and prompt anthelmintic. It has been used somewhat in human practice, against all kinds of worms. No doubt exists as to its efficiency, although its absolute safety is questionable.

Hall and Foster (1) found that it did not show up very well in animal experiments.

19. COPPER SULPHATE (class I). 0.001:100 = F within 1 day; 0.0001:100 = N for 1 day.

All the dilutions produced considerable agitation on immersion. 0.01 per cent was fatal within 13 minutes.

0.001 per cent; the worms were alive after $2\frac{1}{2}$ hours, but were found dead and flaccid after a day.

0.0001 per cent; after a day, the worms showed good tone, although they were very inactive, even on stimulation.

The toxicity of the copper salt is very high, being equalled only by mercuric chlorid, and surpassed by no other drug, as far as tried. Hall and Foster (1) note its use against stomach

worms in lambs and found it highly efficient. They found it unsuitable for dogs, because of the emesis; and this would apply with still more force in man. Investigations with non-emetic compounds might be worth while.

The high efficiency of the copper sulphate may make it available as a "worm killer" for walks, lawns, etc.

20. EPINEPHRIN. This was tried for the possible physiologic effects, rather than as an anthelmintic. 0.0005:100 produced no visible effect in a day. No further experiments were made.

21. ETHER (class III?). $\frac{1}{5}$ saturated = F within 1 day; $\frac{1}{20}$ saturated = N for 1 day.

Ether was used as dilutions of a filtered saturated solution made with the aid of chalk.

The saturated solution depressed at once, and was fatal in less than 21 minutes.

The $\frac{1}{5}$ saturated solution also depressed at once, the animals appearing almost dead in 7 minutes and in $\frac{1}{2}$ hour. However, they recovered in air. Those left in the solution (in a tightly stoppered bottle) were found dead and beaded on the next day.

The $\frac{1}{20}$ saturated solution produced no visible effect of any kind for a day.

Ether thus appears a fairly efficient anthelmintic, although it would need to be determined whether an efficient dose is safe.

Hall and Foster (1) observed in dogs a rather slight efficiency against ascaris, none against hookworm, and probably some against tapeworms.

22. FERRIC CHLORID, TINCTURE (class IV). 1:100 = F in 2 hours; 0.1:100 = N for 1 day.

Inasmuch as the alcohol and acidity as well as the ferric chloride are concerned in the effect, the concentrations are given simply in terms of the U. S. P. tincture.

1:100 produced immediately marked agitation; within one hour, the worms were practically dead; in 2 hours they were quite dead and stiff.

0.1:100 showed no effects.

"Tincture of iron" is used locally in enemas against oxyuris. It would evidently be effective if 1 per cent of the tincture is employed.

However, ferric chlorid is not a very powerful anthelmintic. If the entire activity of the tincture is referred to its ferric chlorid, it would bring this into class III.

23. GENTIAN, EXTRACT (class IV). 1:100 = F in 1 day; 0.2:100 = N for 1 day.

Undiluted solid extract. Contact with a rod smeared with the extract caused prompt repulsion.

1 per cent solution in water: slight agitation on immersion; appears normal in an hour; dead and flaccid next day.

0.2 per cent: no definite effect in a day.

Gentian was tried as representing general bitters. It is a weak anthelmintic and would evidently be ineffective by mouth, although it might be efficient locally by enema.

24. GLYCERIN (class V). A 1 per cent solution does not produce any perceptible change in a day. Concentrated glycerin causes repulsion, presumably by osmotic irritation.

25. GRANATUM, active sample (class III). 0.1 per cent = F; 0.01 per cent = N. Old museum sample (class IV). 1 per cent = F; 0.2 per cent = N.

The *active sample* was furnished by Henry Kraemer, and was used in May, 1918, as filtered infusion.

0.5:100 increased the tone and excitability in 2 hours; dead on next day.

0.1:100; found dead and flaccid in 21 hours.

0.01:100 (freshly made); were normal for 2 days.

The *museum sample* was of uncertain age. It was also used as infusion.

1:100 produced violent agitation on immersion and over an hour. In 2 hours, the worms had calmed down. Next day they were dead and of a "cooked" appearance (doubtless coagulated by the tannin).

0.1:100 also produced considerable agitation on immersion and over 1½ hours. Next day the worms appeared normal.

26. HISTAMIN (class II). 0.001:100 produced some agitation on immersion. After 1 day, one worm was dead and beaded, the other four were alive. After 2 days, a second worm had died, leaving three alive.

The rather high toxicity of histamin is interesting in view of its occasional presence in the alimentary canal, for instance, in connection with ptomaine poisoning.

27. KAMALA (class III). $0.1:100 = F$ in 1 day; $0.01:100 = N$ in 1 day.

This was a museum sample of unknown age, used as filtered infusion with a little bilein-bicarbonate.

The tests were made in May.

0.5 per cent was fatal in 2 hours.

0.1 per cent; the worms were lively in 1 hour, dead in 21 hours.

0.01 per cent; the worms were normal in 21 hours.

The results sustain the reputation of Kamala as an active vermicide.

28. KUSSO (class IV). $0.25:100 = F$ in 1 day; $0.05:100 = N$ in 1 day.

A museum specimen of unknown age was used as decoction. Agitation occurred on immersion in all the dilutions.

2.5 per cent killed the worms within 40 minutes.

0.25 per cent killed between $1\frac{1}{2}$ hours and 1 day.

0.05 per cent; nearly normal on next day.

Presumably this specimen has deteriorated from age. Fresh samples would doubtless range with the active anthelmintics of class III.

29. MERCURIC CHLORID (class I). $0.001:100 = F$ in 1 day; $0.0001:100 = N$ in 1 day.

0.001 per cent produced violent agitation, which, however, subsided promptly. In 2 hours, the worms appear almost normal. They are dead on the next day.

0.0001 per cent produced no effect, beyond slight agitation on immersion.

Mercuric chlorid is thus one of the three most active anthelmintics. Schroeder (2) found 0.1 per cent fatal to ascarids within an hour. It cannot be safely used clinically, because of its high toxicity to mammals.

30. MUSTARD OIL (class I). $0.0025 = F$ within $1\frac{1}{2}$ hours; $0.0005 = S$ in 1 day.

The volatile mustard oil was added as a 0.5 per cent solution in 50 per cent alcohol.

0.025 per cent; momentary agitation; then coil up, become depressed, and die within 5 minutes.

0.0025 per cent; immediate strong agitation; slightly depressed in $\frac{1}{2}$ hour; dead in $1\frac{1}{2}$ hours.

0.0005 per cent; immediate considerable agitation; next day, 5 very sluggish; 2 barely alive; 3 dead.

The mustard oil in 0.0005 per cent can be smelled and tasted, but is not irritating to the mouth. The very high toxicity of mustard oil is surprising, although it confirms the anthelmintic efficiency of "spiced" foods. It is interesting that the ham sandwich is so often taken with its antidote.

31. NAPHTHALEN (class II). 0.01:100 = F in 1 day; 0.002:100 = S for 1 day.

The solutions were made with the acid of the bilein-bicarbonate mixture.

In 0.01:100, the worms were practically dead in 3 hours; and quite dead on the next day.

In 0.002:100, they were practically dead in 1 day.

In 0.001:100, they were much depressed in 1 day, but alive.

Schroeder (2) found a saturated solution of naphthalen fatal to ascarids within 15 hours.

It is evidently a very highly effective anthelmintic.

32. ONION, FRESH (class IV). 1 per cent = F; 0.2 per cent = practically F; 0.1 per cent = N.

Fresh onion was macerated in water and filtered.

In 1 per cent, the worms appeared normal in $3\frac{1}{2}$ hours, but were found dead on the next day.

In 0.2 per cent, they were also normal in 3 hours, and practically dead on the next day.

In 0.01 per cent, they appeared quite normal for a day.

Fresh onion is therefore a fairly efficient anthelmintic, supporting the usefulness of the raw onion and lettuce supper as a preparative for the more toxic anthelmintics.

Darwin (7) found that earthworms are fond of onions as food. Perhaps however, their fondness is satisfied by small quantities;

or it is also possible that the oil had been practically leached out of the onion leaves employed by him.

33. PARADICHLORBENZENE (class II). 0.01:100 = F; 0.001:100 = N.

The substance is nearly insoluble in water, so that a watery solution made with 0.01 per cent produced no change.

When the solubility was increased by the bilein-bicarbonate combination, 0.01 per cent proved fatal in less than 20 hours.

0.001 per cent causes no change in one day; the worms were dead in 2 days.

My attention was called to this substance by an article by Konantz (10). I am also indebted to him for the specimen. The substance is evidently a powerful anthelmintic, and should be very useful, if it is found safe. This very important point remains to be determined.

34. PELLETIERIN TANNATE (class II). 0.005:100 = F; 0.0025:100 = N.

Specimens. Four samples were used, three of these having been examined chemically by L. E. Warren.

Method. It was found that these specimens did not dissolve perfectly in water or in bilein-bicarbonate solution. After some preliminary tests, these were abandoned and the following standard method evolved: The pelletierin tannate, 0.02 gram is dissolved in 100 cc. of distilled water by the aid of 0.2 cc. of 1 per cent HCl, with thorough trituration in a mortar. This stock solution, which must be freshly made, is then diluted as follows:

40 cc. of stock solution plus 40 cc. of water equals 0.01 per cent of pelletierin tannate.

20 cc. of stock solution plus 60 cc. of water equals 0.005 per cent of pelletierin tannate.

10 cc. of stock solution plus 70 cc. of water equals 0.0025 per cent of pelletierin tannate.

5 cc. of stock solution plus 75 cc. of water equals 0.00125 per cent of pelletierin tannate.

There is considerable agitation on immersion; but the final results are counted after one day. Only the limit concentrations need be given in this paper.

ORIGIN OF SAMPLE	FATAL	PRACTICALLY FATAL	NORMAL
Sample I, an old Lehn and Fink specimen	0.01		0.005
Sample II, M. C. W.....	0.01	0.005	0.0025
Sample III, P. W. R.....	0.01	0.005	0.0025
Sample IV, Merck.....	0.005		0.0025

It will be noted that pelletierin tannate is, in fact, a very powerful anthelmintic, about half as strong as oleoresin of aspidium; that the commercial specimens are fairly uniform; and that they do not deteriorate easily.

Schroeder (2) found the hydrochlorid harmless to ascarids even in 0.4 per cent concentration.

Hall and Foster (1) found it unsuccessful against tapeworms in cats, but successful in dogs.

35. PELLETERINE TANRET. 10 per cent = F; 5 per cent = S; 1 per cent = N.

This proprietary worm-killer is marketed as a syrup, which forms clear solutions with water. These following dilutions were employed:

10:100; in an hour, the worms are curled and depressed; in a day, they are dead and nodular.

5:100; in an hour, they have massed themselves as a ball at the bottom of the glass. After a day, one is dead, the other four are curled and much depressed.

1:100; they are somewhat depressed in $2\frac{1}{2}$ hours; in a day they are alive and curled up.

Pelletierine tanret is essentially a secret preparation. The advertising pamphlet, while skillfully evading a direct statement creates the suggestion that it is a solution of a salt of pure pelletierine, freed from the other alkaloids of pomegranate.

According to the above results, the anthelmintic efficiency of the tanret syrup is only $\frac{1}{1350} (= \frac{0.0037}{5})$ of that of the commercial dry pelletierin tannate. According to L. E. Warren (13) the ratio of alkaloidal content is about 1:25 (0.818 per cent in the tanret syrup; 17.4 to 22.8 per cent in the dry tannates). This would indicate that the efficiency of the tanret alkaloids is consid-

erably lower than would correspond to their percentage. This contradicts the contention of the advertisers that their preparation is superior, in that they claim to have eliminated inactive alkaloids from the commercial pelletierin.

The usual dose of the tanret syrup is given as the contents of a bottle, about 30 cc. containing about 0.25 gram of free alkaloids. The average dose of the official pelletierin tannate is also stated as 0.25 gram, but would correspond to 0.05 gram of free alkaloids, i.e., $\frac{1}{5}$ of the amount in the tanret dose. This represents just about the difference in anthelmintic efficiency.

In brief, then, the official dose (0.25 gram) of any of the commercial brands of the pharmacopeial pelletierin tannate, dissolved in 30 cc. (an ounce) of syrup of citric acid, would be just as pleasant, much cheaper, and probably about four times as effective as the average dose of the secret pelletierine tanret.

36. PEPPER, BLACK (class IV). 0.125:100 = F; 0.1:100 = S.

The freshly powdered drug was infused (0.5 per cent), with bilein-bicarbonate, filtered and diluted with water.

0.5 per cent = dead and diffuent in $2\frac{1}{2}$ hours.

0.25 per cent = normal in 1 hour; dead and flaccid next day.

0.125 per cent = nearly dead in $3\frac{1}{2}$ hours; dead and flaccid next day.

0.1 per cent = after a day, very much depressed and flaccid, but alive.

Pepper is another instance of the fair anthelmintic value of the "sharp" condiments. It will be discussed further under synergistics.

37. PHYSOSTIGMIN. 0.0006 per cent = S.

This was not tried for toxicity, but for physiologic effects. The results were quite curious. When placed in the 0.0006 per cent solution, there was some immediate agitation. On the next two days, the worms were found tightly curled in s form, and unable to straighten out.

38. PICROTOXIN. 0.01 per cent = S.

A 0.01 per cent solution was made in bilein-bicarbonate. The worms appeared normal in an hour. After a day, they were much more depressed, clubshaped, but alive.

Schroeder (2) found 0.1 per cent picrotoxin harmless to ascarids in 24 hours.

39. PIPERIN (class III). 0.01 = S.

A 0.01 per cent solution in bilein-bicarbonate produced depression in an hour. After a day, the worms were flaccid and practically dead.

Piperin doubtless plays an important part in the toxicity of pepper but since its toxicity is only ten times as great, the oil, etc., must be the more important ingredients.

40. POTASSIUM CYANID (class III). 0.03:100 = F; 0.01:100 = N.

A pure sample (Schuchardt) was used.

0.1 per cent: normal in 6 minutes; slightly depressed in 30 minutes; next day dead and beaded.

0.03 per cent: somewhat depressed in $2\frac{1}{2}$ hours. After 5 hours almost paralyzed, but move. After 1 day same. Transferred to pure water, but dead when seen on fourth day.

0.01 per cent: no effect immediately, or after $\frac{1}{4}$ hour and after one day. Perhaps rather sluggish.

The low toxicity of cyanid is in harmony with the long life of the worms under stale water. Evidently, their oxidation is low. Ascarids are also resistant. They die after $1\frac{1}{4}$ hours in 3 per cent KCN, but the effects are rather those of the alkali.

41. PUMPKIN SEED, FRESHLY PEELED (class III). 0.1 per cent = F; 0.01:100 = N.

The specimen used was germinable "Yellow Connecticut Field Pumpkin," bought in May. The seeds were peeled, crushed, and rubbed with 100 or 1000 parts of water and macerated overnight. They were then used, with or without filtration through paper, as follows:

Unfiltered suspensions.

1 per cent; normal in one hour, found dead in 21 hours.

0.1 per cent; after 21 hours, 3 are found dead, 2 just alive.

0.01 per cent; normal after 1 day, dead and diffuent in 2 days.

Filtered solutions.

1 per cent; normal in 1 hour, found dead in 21 hours.

0.1 per cent; found dead in 21 hours.

Old pumpkin seeds. 2:100, S in one day.

A museum specimen of uncertain age (but at least 5 years old) was used.

Ten grams yielded 7.3 grams of shelled seed.

Two per cent of shelled seed was crushed and macerated in water for one day, and filtered. After one day, the worms were greatly depressed, but alive.

The experiments vindicate the old and somewhat discredited confidence in this cheap and highly efficient, and presumably harmless anthelmintic. It should be reëxamined clinically. Inefficient results may have been due to the use of aged drug, which is shown to lose greatly in activity. The subject is further discussed under squash seed.

42. QUASSIA EXTRACT (class IV). 0.5 per cent: 100 = S for 1 day; 0.05: 100 = N for 1 day.

The official quassia extract was dissolved in water. With 0.5 per cent the worms showed very slight depression in 10 minutes, and in 2 hours. After a day, they were alive, but exhibited very slow conduction; only a part of the body contracting.

With 0.05 per cent no effect was observable in a day.

Quassia was tried because of its use in enemas against oxyuris. Apparently it is a very weak anthelmintic, as are also all other simple bitters that were tried (Gentian, picrotoxin, quinin, and strychnin).

43. QUININ HYDROBROMID (class III). 0.1: 100 = F in 3 hours; 0.02: 100 = N for 1 day.

0.1 per cent produced immediately considerable agitation. When inspected after 3 hours, the worms were dead.

0.02 per cent caused very slight agitation immediately. In 15 minutes, the animals appeared normal; also on the next day.

When a trituration of quinin and chalk is placed at the bottom of a conical glass of water, the worms tend to draw away from it.

The toxicity of quinin is rather low, considering its toxicity to other lower animals. The apparently sluggish metabolism of the worms may furnish an explanation. Schroeder (2) found ascarids even more resistant; they appeared normal after 4 hours in quinin hydrochlorid.

44. SACCHARIN SODIUM (class V). 1: 100 = S.

Saccharin was dissolved by addition of one-half its weight of sodium bicarbonate. The tests were made in May:

1 per cent of saccharin with $\frac{1}{2}$ per cent of bicarbonate: after 1 day, two worms are almost dead. Five others are depressed, but quite alive.

0.2 per cent of saccharin with 0.1 per cent of bicarbonate: apparently normal in one day.

Saccharin is practically not anthelmintic, although it is not quite indifferent.

45. SACCHAROSE. Indifferent.

In 1 per cent the worms behave normally at the end of a day. When placed on solid granulated sugar, they burrow into it, in very striking contrast to their violent withdrawal from crystallized sodium chlorid. The worms therefore are rather unsuspceptible to osmotic irritation.

46. SANTONIN IN BILEIN BICARBONATE (class II). 0.01:100 = F in 1 day; 0.001:100 = N in 1 day.

Santonin is very sparingly soluble in water. Several solvents were tried. The best results were obtained with the bilein-bicarbonate mixture, which was intended to imitate the intestinal fluid.

Santonin-bilein bicarbonate. The stock mixture was made with santonin, 0.1; bilein, 0.04; sodium bicarbonate, 1; water 100. The mixture is turbid, and was shaken before using. The dilutions were made with water. When 9 parts of water are added to 1 part of the stock suspension, solution becomes almost complete. The following concentrations are stated as santonin, the other constituents being in the proportions just explained.

0.1 per cent; immediate moderate agitation. After end of a day, one worm is just alive. The other four are dead and diffuent.

0.01 per cent; in 1 hour, the worms are somewhat depressed and are twisted in a ball. After one day, they are found dead.

0.001 per cent; after 1 day, the animals are found alive, but curled and depressed.

Santonin bicarbonate. A mixture of santonin, 0.1 gram, sodium bicarbonate, 1 gram and water, 100 cc. Considerable remains undissolved, but is left in the mixture. Worms placed in the sus-

pension (0.1 per cent santonin) immediately show agitation. After a day, they are found dead, flaccid and nodular.

Santonin bilein. Santonin, 1 gram; bilein, 1 gram, and water, 100 cc.; gives a turbid suspension. This is still slightly cloudy when one part of the suspension is diluted with 19 parts of water. The following experiments were tried.

Santonin and bilein, each 0.05 per cent: immediately, fair agitation; $1\frac{1}{2}$ hours relaxed, almost dead; 1 day, dead and flaccid.

Santonin and bilein each 0.005 per cent: practically normal for one day.

These results show a very high toxicity (class II) in the presence of bilein, which acts presumably as a solvent, but possibly by favoring the penetration of the drug. The relatively poor results of the older observers (Schroeder and Trendelenburg) are probably attributable to the absence of the bile, i.e., to incomplete solution. "Sodium santoninate" does not possess this high toxicity.

Hall and Foster (1) found santonin rather disappointing in dogs and not very practical in pigs. The explanation of their poor results is not clear in view of the undoubted clinical efficiency in human subjects. We shall recur later to the mechanism of actions, etc.

47. SANTONINATE OF SODIUM (class IV). 0.5:100 = F, practically; 0.2:100 = N.

Solutions (1 per cent) are neutral to litmus. The solid salt is strongly repulsive to the worms.

1 per cent: immediately there is very considerable agitation, and the worms appear rather stiff. In $2\frac{1}{2}$ hours, they are apparently dead, but move feebly. After 1 day, 3 were found dead, the 2 other almost dead, the anterior half of the body apparently stiff, whilst the posterior half can move.

0.5 per cent: immediately slight agitation, $1\frac{1}{4}$ hours depressed; 1 day, relaxed and almost dead, but respond slightly.

0.2 per cent: no apparent effect for 1 day.

48. SAPONIN (class III). 0.1:100 = F in 1 day; 0.01:100 = N in 1 day.

The experiments were made mainly for possible physiologic interest. Merck's "pure saponin" was used.

0.1 per cent; depressed and sluggish in 1 hour; after 1 day found dead and diffluent.

0.01 per cent; for 1 day, somewhat sluggish and curled, but practically normal.

49. SOAP (class III). 0.1:100 = F within 45 minutes; 0.03:100 = N, practically, for 1 day.

Powdered castile soap was used.

0.1 per cent produced immediate agitation. When inspected after 45 minutes, the worms were dead and slimy.

0.03 per cent also produced immediately considerable agitation. After 10 minutes they were nearly normal, perhaps slightly depressed. No further change had occurred in 3 hours and in 1 day.

0.01 per cent produced practically no effect.

Soap was investigated because of its local use in enemas against oxyuris. It would evidently be highly effective. In view of the rather low toxicity of saponin and of bile salts, the high efficiency of soap is evidently not due to lipolysis, but rather to the alkalinity. In fact, the toxicity of soap is about the same as that of sodium carbonate.

50. SODIUM BICARBONATE (class V). 1:100 = N.

Powdered sodium bicarbonate produces repulsion. No effect whatever was noted from a 1 per cent solution in one day, and from a 0.2 per cent solution in 4 days.

The harmlessness of sodium bicarbonate in concentrations of 1 per cent and less renders it a suitable solvent for difficultly soluble acids, such as saccharin.

51. SODIUM CARBONATE CRYSTALS (class III). 0.1:100 = F within 35 minutes; 0.03:100 = S for 1 day.

The undissolved crystals are strongly repulsive to the worms. 1 per cent; within 18 minutes the worms are dead and slimy.

0.1 per cent; immediate slight agitation. In 35 minutes, the worms are found dead.

0.03 per cent; immediate slight agitation. In $2\frac{1}{2}$ hours, they appear normal. In 1 day, they are weak, but alive.

0.01 per cent; no visible effect within a day.

Sodium carbonate is therefore a quite strong anthelmintic, provided that it comes in direct contact with the worms.

Crozier (11) has investigated the sensory reaction of earthworms to alkalis in detail. He found that NH_4OH is much more active than equimolecular NaOH ; presumably because of easier penetration.

Ascarids have a relatively high resistance to sodium carbonate, 5.8 per cent requires 5 hours to be fatal (Schroeder, 2). This is presumably due to the resistance of the chitinous envelope. Schroeder found sodium hydroxid 0.2 per cent fatal in 20 minutes, whilst 0.022 per cent produced no effect.

52. SODIUM CHLORID (class V). 1 per cent = N for 1 day.

Dry crystals and presumably strong solutions cause repulsion. A 1 per cent solution had no effect in one day.

53. SPIGELIA (class IV). 0.5:100 = F; 0.1:100 = nearly F; 0.02:100 = N.

An active specimen was obtained from Kraemer, and used as filtered infusions in May.

In 0.5 per cent, the worms were practically dead in 2 hours. When inspected in 25 hours, they were dead.

In 0.1 per cent they were just alive in 21 hours.

In 0.2 per cent they remained normal during two days.

An old museum specimen gave nearly identical results:

In 0.5 per cent the worms were almost dead in 2 hours, but retained a good tone. In 1 day, they were all dead, the tails filiform.

In 0.1 per cent they remained normal during the day.

These results indicate that spigelia has a demonstrable anthelmintic action, but so weak that it would be quite unreliable. This agrees well with the modern clinical estimate. Hall and Foster (1) found it practically inefficient in dogs.

54. SQUASH SEED, "HUBBARD," FRESH PEELED (class III). 0.1:100 = F in 1 day; 0.01:100 = F in 1 day.

These experiments were made on germinable seed, purchased in a seed store and tested in May. The peeled seeds were crushed, rubbed with 20 parts of water, macerated overnight, and then diluted, with or without filtration. Exactly the same results were obtained, so that the active constituents are not removed by filtration. The results were as follows:

1 per cent; normal in 1 hour, found dead in 21 hours.

0.1 per cent; found dead in 20 hours.

0.01 per cent; normal for 2 days.

Effect of boiling. This was investigated in an earlier series. The seed was taken directly from the squash. 30 grams of the fresh seed yielded 10.25 grams of shelled seed. The latter was used as a macerate, filtered, with and without previous boiling.

<i>Unboiled filtrate</i>	<i>Boiled filtrate</i>
0.4 per cent = F in 1 day	2 per cent = F, practically in 2 days (3 dead, 2 almost dead)
0.08 per cent = N for 1 day	1 per cent = N, practically for 1 day.
	0.4 per cent = N for 1 day

Squash seed like pumpkin seed, is evidently a quite powerful and harmless anthelmintic. The active constituents appear to be soluble in water, and could be administered as solutions or as an unfiltered "milk." They are gradually destroyed by boiling. They probably also diminish with the aging of the seeds and this may be responsible for the unreliable results that seem to have been sometimes obtained.

The unlimited supply, the low cost and the harmlessness of the pumpkin and squash seeds should revive clinical interest, especially at this time when other active anthelmintics are scarce.

55. STRYCHNIN SULPHATE. 0.01:100 = N.

This was used for its physiological interest. In the 0.01 per cent solution, the worms became very lively, and curled up, similar to physostigmin, but not as much. They were observed in $\frac{1}{2}$ hour and 1 day. Schroeder (2) found that 0.5 per cent solution of strychnin salt left ascarids normal over three hours.

56. TANNIN (class III). 0.05:100 = F in 1 day; 0.01:100 = N for 1 day.

Solid tannin produces immediately strong repulsion. All the solutions also cause immediate agitation.

0.1 per cent; normal in an hour; dead and stiff in a day. The worms killed by this and the following solution show a curious protrusion of the mouth.

0.05 per cent; similar to the preceding.

0.01 per cent; except for the immediate agitation, the worms show no effect for two days.

Tannin is a fairly strong anthelmintic, which may be quite useful for enemas. It would, of course, be useless by mouth.

57. THYMOL (class II) in BB., 0.01:100 = F; 0.002:100 = S; 0.0005:100 = N; $\frac{1}{10}$ saturated solution = F; $\frac{1}{30}$ saturated solution = N.

Thymol was tried by two methods, i.e., as dilutions of a saturated solution with the aid of chalk trituration, and by trituration (0.01 per cent) with the bilein-bicarbonate mixture. All the solutions produced immediately considerable agitation.

The chalk-trituration gave the following results:

Saturated solution: Fatal within $\frac{3}{4}$ hour.

$\frac{1}{10}$ saturated solution; in 1 hour, very flabby, but move. After 1 day, dead.

$\frac{1}{50}$ saturated solution; normal in $1\frac{1}{2}$ hours and 1 day.

The bilein bicarbonate solutions gave the following:

0.01 per cent; practically dead in $2\frac{1}{2}$ hours.

0.002 per cent; still somewhat agitated after 1 hour. After 1 day, practically dead.

0.0005 per cent; considerably depressed and sluggish but not fatal in 1 day.

The results show that thymol is a very active vermicide, in agreement with the clinical experience. Hall and Foster (1) found it of rather variable efficiency in dogs.

58. TURPENTINE OIL (class II?). $\frac{1}{10}$ saturated solution = F in 1 day; $\frac{1}{30}$ saturated solution = N for 1 day.

This was used only as dilutions of a saturated solution made with the aid of chalk.

The $\frac{1}{10}$ saturated solution produced immediately considerable agitation, lasting over an hour. Next day, four worms were dead, flaccid and beaded. One was alive.

The $\frac{1}{30}$ saturated solution produced no marked effects during a day.

Turpentine is thus a very powerful anthelmintic; but its toxic actions interfere with the use of efficient doses. This was also the conclusion of Hall and Foster (1).

TYRAMIN. 0.02: 100 = N for 1 day.

This was tried for its possible physiological interest. The above concentration caused slight temporary agitation, but no other effect was observable for a day.

COMBINATIONS OF ANTHELMINTICS

The usual preparatory treatment with spices gives the opportunity of combining their toxicity with that of the more active anthelmintics. The use of pumpkin seed as an adjuvant offers similar opportunities. The active anthelmintics are not often combined in modern practice. Such combinations would be desirable, however, should it be found that the toxicity to the worms is additive; for the toxicity to the mammalian host is often based on different actions, so that it would not be additive. These considerations prompted the following experiments. All were made with filtered bilein-bicarbonate solutions.

Stock solutions. These were mixtures of equal parts of the just fatal concentrations of both drugs. They would therefore contain one-half of the fatal concentration of each constituent.

The stock solution should be just fatal if there were simple summation of the toxicity of both drugs; subfatal if there were only partial summation; and superfatal (i.e., capable of dilution) if there were potentiation.

The results will be presented in this form, i.e., as the toxicity of the stock mixtures, containing 50 per cent of the fatal concentration of each of the two drugs.

60. PEPPER AND ASPIDIUM OLEORESIN. Fatal concentration = pepper 0.125 per cent; aspidium oleoresin (III) 0.002 per cent.

Stock solution = pepper, 0.0625 per cent; aspidium oleoresin (III), 0.001 per cent.

Theoretical toxicity = 100.

Results: stock solution = F; $\frac{1}{2}$ stock solution = F; $\frac{1}{6}$ stock solution = N, practically.

Since a $\frac{1}{2}$ stock solution is fatal, the toxicity is at least twice the summed toxicity of the ingredients. There is, consequently actual summation.

61. PEPPER AND CHENOPODIUM OIL: Stock solution = pepper, 0.0625 per cent; chenopodium oil, 0.005 per cent.

Results: Stock solutions = F; $\frac{1}{2}$ stock solution = F; $\frac{1}{6}$ stock solution = S (much depressed, but alive).

Again, as in No. 60, there has been a potentiation of at least 100 per cent.

62. PEPPER AND THYMOL. Stock solution = pepper, 0.0625 per cent; thymol, 0.001 per cent.

Results: Stock solution = F; $\frac{1}{2}$ stock solution = F; $\frac{1}{6}$ stock solution = S, much depressed, but alive.

As with aspidium and chenopodium, the pepper has not only summed, but more than doubled the toxicity.

63. PEPPER AND SANTONIN. Stock solution = pepper, 0.0625 per cent; santonin, 0.001 per cent.

Result of stock solution = F. There is at least full summation; the problem of potentiation was not investigated.

64. CHENOPODIUM AND THYMOL. Stock solution = chenopodium oil, 0.005 per cent; thymol, 0.001 per cent.

Result of stock solution = F just: 4 worms are dead, 1 barely alive. Consequently, there was just summation.

65. CHENOPODIUM AND ASPIDIUM. Stock solution = chenopodium oil, 0.005 per cent; aspidium oleoresin (III), 0.001 per cent.

Result of stock solution = just F, i.e., practically dead. This corresponds to simple summation.

66. THYMOL AND ASPIDIUM. Stock solution = thymol, 0.001; aspidium oleoresin (III), 0.001.

Result of stock solution—just fatal, i.e., practically dead. This again corresponds to simple summation.

67. CHENOPODIUM AND SANTONIN. Stock solution = chenopodium oil, 0.005 per cent; santonin, 0.001 per cent.

Results of the stock solution = just F, i.e., practically dead. This is simple summation.

68. ASPIDIUM AND SANTONIN. Stock solution = aspidium oleoresin (III), 0.001 per cent; santonin, 0.001 per cent.

Result of the stock solution = F, i.e., full summation.

69. THYMOL AND SANTONIN. Stock solution = thymol, 0.01 per cent, santonin, 0.001 per cent.

Result of stock solution = F, i.e., full summation.

The combination of the active anthelmintics (Aspidium, chenopodium, santonin, and thymol) with each other gives full summation. The combinations of any two of these permits the dose of each to be reduced to one-half. Combination with pepper gives not only complete summation, but actual potentation of the effects.

"REPULSIVE EFFECTS" OF ANTHELMINTICS

The apparent discrepancy between the low experimental vermicide efficiency and the high clinical efficiency of santonin led to various attempts to explain its effects by a "vermifuge" action. The present investigation has removed the need for these rather forced explanations in that it has shown that santonin is highly toxic to worms in the presence of a suitable solvent, i.e., bile salts. However, the experiments on "repulsive" effects are still interesting, since repulsive effects doubtless do occur, and may have more or less to do with the clinical efficiency; although they are not at all specific for santonin, but are produced by practically all vermicides.

Schroeder (2) observed that ascarids are put into much more active movement by santonin. This he interprets as a sign of discomfort, which drives the worms into the colon, from which they are then expelled by the cathartic, in a living condition.

These active movements apparently correspond to the "agitation" observed in earthworms when they are first placed in almost any toxic solution. Trendelenburg (5) showed that the stimulant effect occurs also in ganglion-free preparations of the earthworm, and considers that this effect is at least quantitatively characteristic for santonin. However, it is also given by chenopodium oil and evidently also by other poisons.

Schultz (12) also believes that anthelmintics produce "rapid vermiform movements" in the parasites; if their irritation is sufficiently great the worms will attempt to escape. If the concentration rises above the stimulant concentration, the worms become paralyzed.

The irritant, agitative, "repulsive" and "escape" movements can be excellently observed in the simplest manner on earth worms. They are evidently of the same type as those of the parasitic worms, and much more easily studied and understood. Several arrangements are suitable for these observations.

Agitative effect. When worms are placed in water in a conical glass, they generally assume a vertical position, head down, and body waving gently. When an irritant is added, the movements become much more violent, or even whiplike. Doubtless worms in this state of incoordinated movement would be unable to resist the peristaltic sweep.

Repulsive effect in conical glasses. If the irritant is introduced in the bottom of the glass, it comes in contact with only a part of the worm (usually the head). This is promptly withdrawn or snapped back into the unpoisoned stratum. The worms may thus "withdraw" a small distance from the irritant; where it comes to rest. This occurs with sodium santoninate but equally well with sodium chlorid—in fact, with any irritant. In the intestines, it would tend to drive the worms down the intestines a short distance ahead of the irritant.

Repulsive effects studied on leaf mold. This natural habitat of the worms is also suitable to illustrate their "withdrawal" or "repulsion" by irritation or toxic substances. It is easy so to arrange the leaf mold that parts of numerous worms are exposed, practically at rest. These are sprinkled with a little of the dry substance or solution. The following observations were made:

Powdered chalk. This produces only a momentary, mechanical reaction. Chalk saturated with *aspidium* or *chenopodium* causes immediate extensive withdrawal, and violent whiplike movements. Dry *bilein* also produces a whip reaction.

Kousso, 2.5 per cent solution gives immediate reaction from the head, apparently less from the tail. *Quassia*, $\frac{1}{2}$ per cent; solution of solid extract gives no reaction. Fresh *onion* prompts immediate escape.

Dry *santonin*, *sodium chlorid* and *sodium bicarbonate* cause immediate withdrawal. Dry *saccharose* gives only a momentary mechanical reaction which stops at once.

Repulsive effect on blotting paper. When the worms are crawling over the paper, a drop of the reagent is placed in their path:

Bilein-bicarbonate has no effect.

Aspidium (0.03 per cent in BB) causes deflection of the course.

Pepper (0.125 per cent in BB) causes deflection and more energetic movement.

Sodium santoninate or *mercuric chlorid* cause the worm to jerk back at once.

Glass spiral experiment. This experiment was invented by Straub (Fuehner, p. 44) to show the repulsive effect of santonin on ascarids. The intestines are supposed to be represented by a glass spiral, filled with warm Bunge's fluid. The ascarids tend to settle to the bottom of the tube. A strong solution of sodium santoninate is then introduced near the worms, and they generally move away as the santonin diffuses into the solution.

The same phenomena can be demonstrated with earthworms placed in such a spiral filled with water. When a crystal of sodium santoninate is introduced near to the worms, their movements become more lively, and generally carry them some distance away; but as the santoninate becomes diluted by diffusion, the worms tend to gravitate to their old position.

Again, the phenomenon is not at all characteristic of santonin, but is shared by any irritant, and can be demonstrated much more strikingly by adding a few drops of 1 per cent mercuric chlorid. With this, the worms will not only withdraw, but they will be killed and coagulated a little distance (for instance 2 inches) from their original position.

Zigzag tube. In the glass-spiral experiment of Straub, the movements of the worms are complicated by the effects of gravity. It was sought to avoid this by using a zigzag tube, about 1 cm. internal diameter, with eight bends of about 45 degrees about every 10 cm.; the ends being bent vertically upward. The tube was filled with water, and a few worms were introduced. It was hoped in this way that even minor repulsions by substances introduced at one end would be indicated by the distribution of the worms after a considerable time, say 24 hours. It was found however that the normal wanderings of the worms were rather

capricious, so that more reliance was placed on the immediate reactions. The average distance of unpoisoned worms from the starting point was as follows:

	20 MINUTES	40 MINUTES	3 HOURS	6 HOURS	24 HOURS
Experiment 1.....		3.5	10	20	28
Experiment 2.....	7.5	12.0			
Experiment 3.....	14.0		40	33	33
Average.....	11.0	8.0	25	27	30

Santonin. This has a distinct repellent action when placed in a solvent medium (bilein-bicarbonate). In water, the effect is almost nil. The following is perhaps the most instructive experiment.

A fair sized worm is placed in the tube filled with bilein-bicarbonate solution. It is slightly restless. Some santonin crystals are added. The worm jerks back on touching the crystals, some of which adhere to the mucous coating of the skin. The worm then wanders promptly to the other end of the tube, the crystals still adhering. A few crystals of santonin are now added to this end, the worm tries to come to the surface of the water, but in doing so must pass the santonin crystals. This causes him to sink back immediately. This manoeuver is repeated several times.

Another worm is added and this also shows the same phenomena.

Santonin-adhesion phenomenon. Attention was called to the adhesion of the santonin crystals to the skin of the worm by mucous threads. Chalk or cane sugar failed to show this adhesion. Santonin shows it only in the presence of sodium bicarbonate; it adhered in 1 per cent sodium bicarbonate, but did not in 0.04 per cent bilein without bicarbonate.

Aspidium oleoresin. This is also markedly repellent. A worm is placed in the tube filled with water, and some aspidium-chalk powder is added; the worm draws back sharply when its head comes within 5 cm. of the powder. Gradually it withdraws beyond the first bend, occasionally attempts to come back, but al-

ways turns around and withdraws when it comes within 5 to 7 cm. of the powder.

Another tube is filled with water, five worms added, some aspidium chalk sifted in, and the average position of the worms observed. This is contrasted with the normal averages as follows:

Average migration of worms

TIME	IN WATER ALONE	WITH ASPIDIUM
	<i>centimeter</i>	<i>centimeter</i>
40 minutes.....	8	10
3 hours.....	25	18
24 hours.....	30	65 (worms lively).

Pelletierin tannate. This is also actively repellent. The tube is filled with water, a worm placed in one end, and a little pelletierin tannate powder is added, forming a small lump. The worm jerks back sharply whenever its head touches the tannate, then turns around and creeps away. It often tries to come back, but always turns away as soon as it comes within about 5 cm. of the pelletierin. Finally, the worm settles down into a ball, at about this distance.

Granatum. This acts similar to the pelletierin. It was tested by the average migration of the worms.

TIME	AVERAGE NORMAL MIGRATION	AVERAGE MIGRATION AFTER ADDING 2 CC. OF 2 PER CENT INFUSION OF GRANATUM
	<i>centimeter</i>	<i>centimeter</i>
20 minutes.....	11	18
40 minutes.....	8	20, in tight wad.
1½ hours.....		same.

Chenopodium oil. This repels like aspidium. When added on chalk, the worm withdraws rapidly to the bend of the tube, and does not approach closer than 7 or 8 cm.

Black pepper. Repulsion; the worm withdraws beyond the next bend. It wanders back and forth, but keeps at least 3 cm. away from the powder.

Onion. Repulsive. A small piece of onion scale repels the worm and keeps it about 2 cm. away.

Mustard oil. Strongly repulsive. A little of a 0.5 per cent solution in 50 per cent alcohol is added: the worm jerks back violently at every approach.

Squash seed. A crushed seed is added. The worm jerks away and escapes. It returns and burrows rapidly into the emulsion; but is evidently very uneasy, turns back, and escapes again.

Copper sulphate. A little of a 0.1 per cent solution is introduced. The worm jerks away sharply on its approach and crawls away. It approaches again, but again jerks away and makes for the other end of the tube.

Tannin. This makes the worm uneasy and causes withdrawal.

Quassia chips. These apparently have little if any effect on the movements of the worm.

Bilein. 2 cc. of 1 per cent added to the upright tube. This causes slight agitation whenever the worm happens to go through the bilein stratum, but it makes no effort to escape.

Hydrochloric acid. Some 1 per cent acid is added, causing violent agitation and death before the worms escape.

Saccharose crystals. These have no effects.

Sodium chloride crystals. The worm jerks back immediately when it is within 2 cm. of the crystals, and then crawl away to the opposite end of the tube.

SUMMARY

All clinical anthelmintics are markedly toxic to earthworms. This simple test may therefore be used for determining whether a given substance has any anthelmintic properties. It may also be used to determine the relative activity of different samples of a given drug. It could not be used to compare the clinical value of different anthelmintics, since this often involves factors other than simple vermicial efficiency; such, for instance, as absorption, local and general toxicity, etc. For instance the highest vermicial efficiency is possessed by substitutes that are not clinically available for this purpose; viz., mercuric chlorid, cupric sulphate, and mustard oil.

Aspidium, chenopodium, pelletierin, thymol, betanaphthol, and chloroform are highly effective. So is santonin in the presence of an appropriate solvent (bile-salts and sodium bicarbonate, simulating the intestinal fluid).

Somewhat less effective, but still quite toxic are kamala, kousso, and granatum. Spigelia is rather feeble.

Fresh (germinable) pumpkin seed and squash seed are quite highly efficient, the active principle being soluble in water and destroyed by boiling. In view of their cheapness, availability, and presumably low toxicity to men, renewed clinical interest in these is indicated.

Spices and "sharp" substances including mustard, pepper, onion and cantharidin are quite toxic. Their use in the preparatory treatment is therefore well justified (except of course cantharidin). Indeed, pepper potentiates or synergizes the effects of the more active anthelmintics.

Mixtures of the active anthelmintics give simple summation of efficiency. This may be useful for decreasing their toxic effects on the hosts. It would need to be determined by further animal experiments and clinical trial.

Oleoresin of aspidium appears to be quite stable, although the dry rhizome deteriorates. Different samples agree fairly in activity. The deposit that occurs in some oleoresins appears to have little if any influence in the activity.

Different samples of pelletierin tannate are also of fairly uniform activity. The "pelletierine tanret" is a secret preparation without any advantage.

Most substances that are toxic to earthworms produce a primary irritation or agitation, that results in the withdrawal of the worm from the neighborhood of the poison. This is observed with santonin, but is no more marked with this than with other anthelmintics.

By virtue of this effect, anthelmintics doubtless often "expel" the parasite when the concentration does not rise sufficiently high to kill the worm.

BIBLIOGRAPHY

- (1) M. C. HALL and FOSTER: J. Agric. Res., 1918, xii, 397.
- (2) H. SCHROEDER: Arch. exp. Path. Pharm., 1885, xix, 291.
- (3) W. STRAUB: *ibid.*, 1902, xlviii, 1.
- (4) BRUENING: Zs. exp. Path., 1906, iii, 564.
- (5) P. TRENDLENBURG: Arch. exp. Path. Pharm., 1915, lxxix, 190.
- (6) S. YAGI: Zs. exp. Med., 1914, iii, 64.
- (7) CHAS. DARWIN: "The formation of vegetable mould through the action of worms."
- (8) CROZIER, W. J.: Am. J. Physiol., 1918, xlv, 323.
- (9) FUEHNER: "Nachweis der Gifte," p. 44 and 45.
- (10) KONANTZ, W. A.: J. Am. Pharm. Assoc., 1918, vii, 341.
- (11) CROZIER, W. J.: Am. J. Physiol., 1918, xlv, 315.
- (12) SCHULTZ, W. H.: J. Am. Med. Assoc., 1911, lvii, 1102.
- (13) WARREN, L. E.: Reports of Chem. Lab. Am. Med. Assoc., x, 60.

XVI. DIFFERENCES IN THE ACTION OF DRUGS ON DIFFERENT PARTS OF THE BOWEL

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It is customary to speak of certain drugs as stimulants or depressants to the intestine, intimating that their effects are uniform from one end of this long organ to the other. To be sure there are a few exceptions. Thus pharmacologists have described local differences in the actions of certain purgatives, some of which are supposed to act particularly on the colon. According to Magnus (1), Pancoast (2) and others morphin causes marked spasm at the pylorus, some slowing in the small intestine and very little change in the colon. Others have felt that morphin acted particularly on the sigmoid (3). Siccardi (4) found that lead acetate stimulates excised segments of small intestine and depresses the colon. Similar observations were made on cats poisoned with lead (5). Bile is said to stimulate the colon but not the small intestine (6). Fatty acids depress the activity of the stomach while they increase that of the small intestine (7). As is well known, adrenalin inhibits the movements of the gastric and intestinal muscles. It is not so generally known that it stimulates the muscle in the last 10 mm. of ileum, including the ileo-cecal sphincter (8). It stimulates also the pyloric muscle as would be expected from Gaskell's theories. He showed that the muscle in the pyloric and ileo-cecal sphincters probably has a different phylogenetic origin from that in the rest of the gut (9). In the fowl the whole duodenum is stimulated by adrenalin; the gizzard is unaffected, and the colon and ceca are inhibited (10).

Salant and his co-workers have shown differences in the actions of a number of drugs on segments from different parts of

the bowel. Thus, sodium tartrate produced a slight decrease in amplitude in the small intestine, and a marked decrease in tone and a stoppage of the contractions in the colon (11). Zinc and nickel salts had more pronounced effects on the duodenum and jejunum than on the ileum and colon (12). Oil of chenopodium was more depressant to the ileum and colon than to the upper small intestine. Caffein caused depression in the ileum while it stimulated the duodenum (13). The writer pointed out in 1914 that the effect of adrenalin was more pronounced on the ileum than on the duodenum (14).

The present work was undertaken with the hope that light might be thrown not only on the pharmacology but on the physiology of the intestine. Other work has convinced me that there are marked differences in the neuro-muscular apparatus in different parts of the tract. It seemed likely that such differences in chemical structure and metabolism would show themselves as differences in reaction to various drugs. Such differences when found might throw light on the nature of the metabolic processes in the various parts of the tract. Thus the graded response to asphyxia, KCN and adrenalin described in a previous paper (15) indicate the presence of a metabolic gradient down the gut.

TECHNIC

Rabbits have been used in all this work. The segments have been taken from the duodenum, the upper jejunum, the middle of the small intestine, the lower ileum about 20 cm. above the ileo-cecal sphincter, and the colon where it parallels the first portion of the duodenum. The beaker contained 400 cc. of Locke's solution kept at 38°C. To insure a prompt and even mixture in the beaker all solid drugs were first dissolved in a little water or dilute alcohol. These substances were then warmed to the temperature of the Locke's solution. The tube through which air was admitted to the solution was drawn out to a fine point and directed horizontally, so that the force of the escaping bubbles would keep up a certain amount of circulation in the beaker. This circulation helped in diffusing the drugs rapidly throughout

the liquid. Moreover, the added substances were always distributed about the edges of the beaker so that no one segment would be overwhelmed at the start by a large dosage.

Segments which had been used and washed were used again mainly for preliminary work in ascertaining the proper dosage.

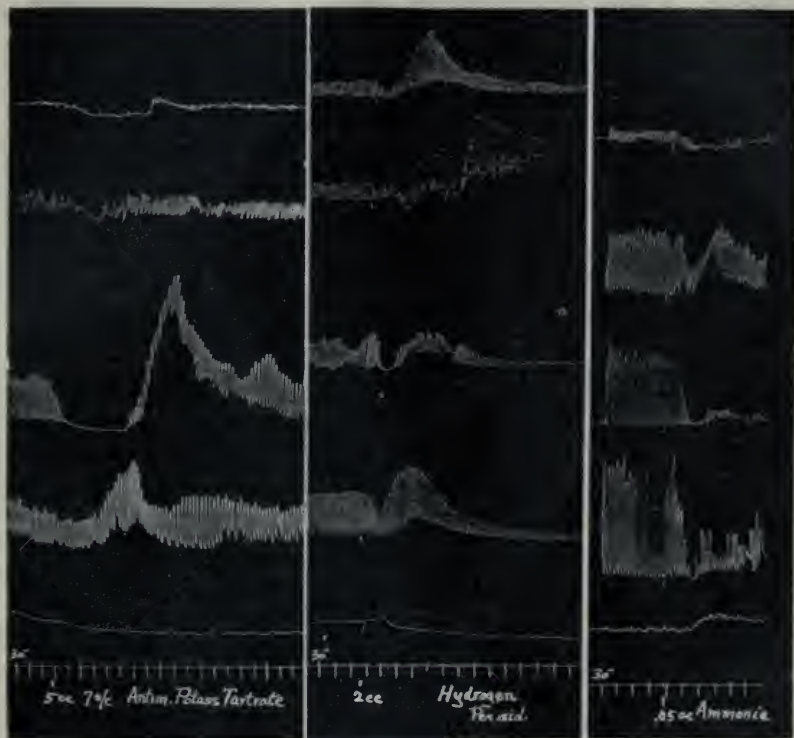


FIG. 1. MIXED DEPRESSION AND STIMULATION

From above downwards in all the tracings the records are from duodenum, jejunum, middle, ileum and colon.

The dose aimed at was the lowest which would produce definite changes in the activities of the segments. After excluding a large number of these preliminary experiments there were 506 left. From 3 to 20 tests have been made with each of 76 different drugs. Some of these drugs are commonly used in medicine as

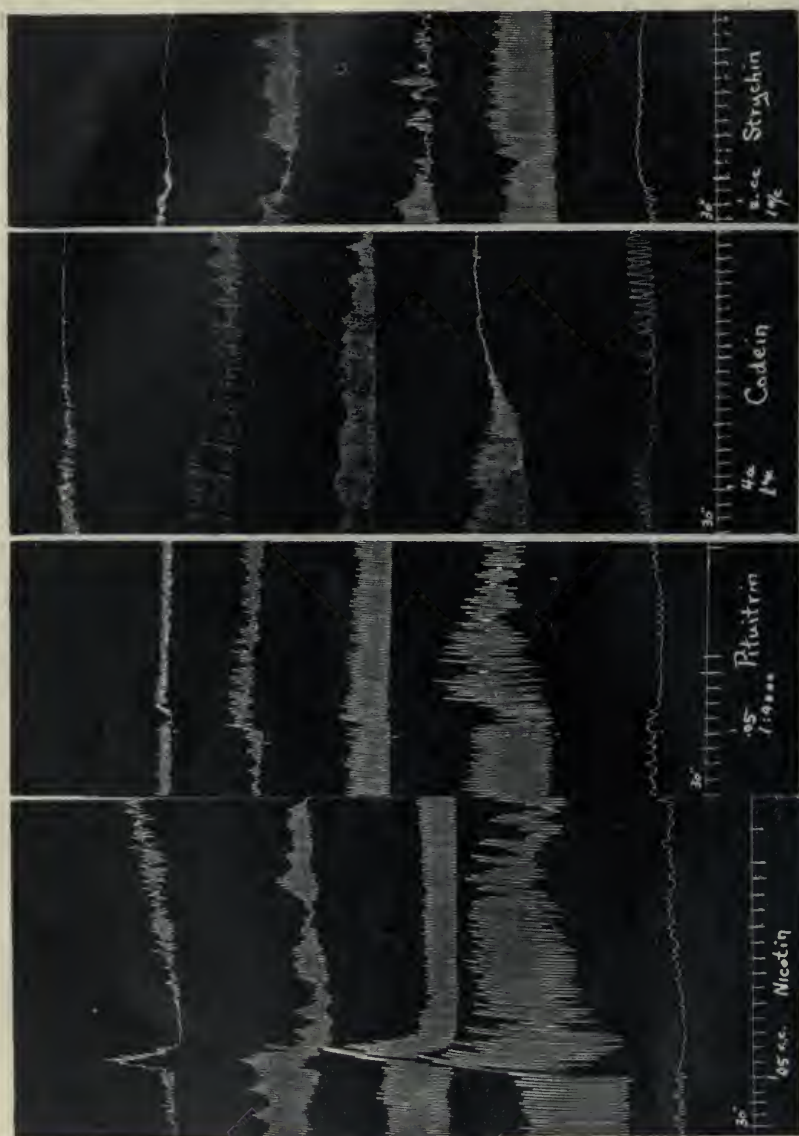


FIG. 2. MIXED DEPRESSION AND STIMULATION

anesthetics, nerve stimulants or depressants; others as purges and emetics. Others are poisons which attack various parts of the protein molecule. For the sake of convenience, these substances will be listed alphabetically. The effects of these drugs on the rate of contraction have been discussed in a previous paper.

Substances which stimulated all parts about equally

Barium chlorid	Potassium chlorid
Eserin	Potassium hydrate
Glucose	Sodium bicarbonate
Mercuric chlorid	Sodium fluorid
Pilocarpin	

Substances which depressed all parts about equally

Acetone	Cocain
Alum ($\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$)	Ether
Anilin	Formaldehyd
Bile (dog's)	Jalap (compound tincture)
Calcium chlorid	Urethane
Calcium lactate	

Substances which showed mixed depressions and stimulations

Some of these drugs stimulate in small doses and depress in large; some depress or stimulate after an initial stimulation or depression; others affect one segment one way and another segment the other way. Following are some protocols showing the results of experiments with a number of drugs which produced these mixed reactions. With the first sixteen the main effect was a stimulant one.

In the following tables -, --, ---, ---- represent varying degrees of depression of tone, amplitude and rhythmicity down to complete stoppage of all movements. +, ++, +++, +++++ represent varying degrees of stimulation. 0 shows that there was no change. -0 indicates a slight depression of short duration; +-+++ indicates a slight stimulation followed by a slight depression and later a marked stimulation. ? indicates doubt as to whether any effect took place, or whether

the rise or drop apparent on the tracings was accidental or due to the drug. +++ "paral." indicates a rise in tone accompanied by a stoppage of the rhythmic movements. "Irreg." shows that the contractions became irregular. "Ampli." shows

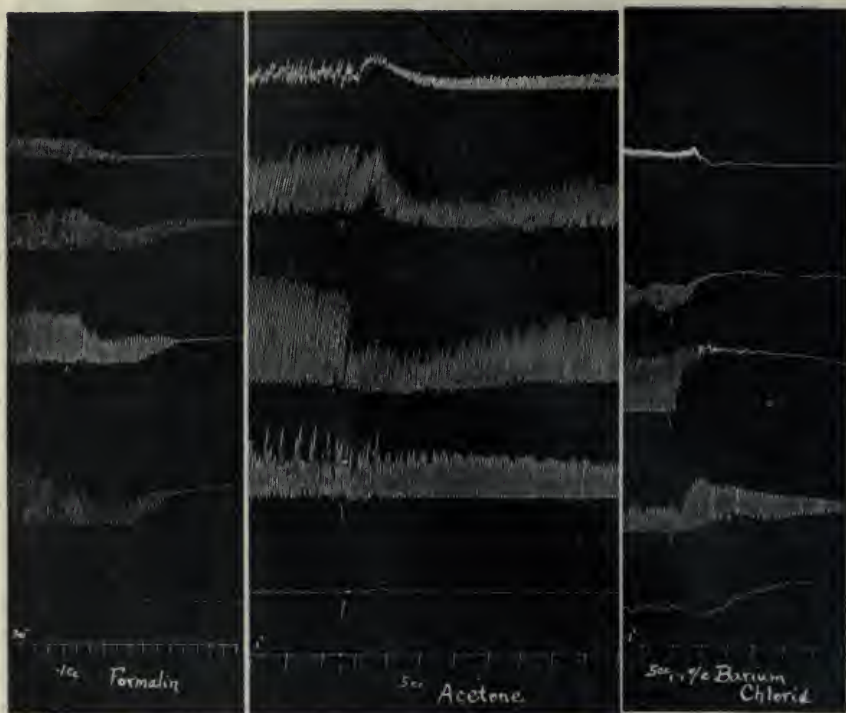


FIG. 3. MIXED DEPRESSION AND STIMULATION

that the change was principally one in amplitude. The letters D., J., M., I., C., designate the segments from duodenum, jejunum, middle, ileum, and colon. The figures in the upper row represent amounts added to 400 cc. of Locke's solution.

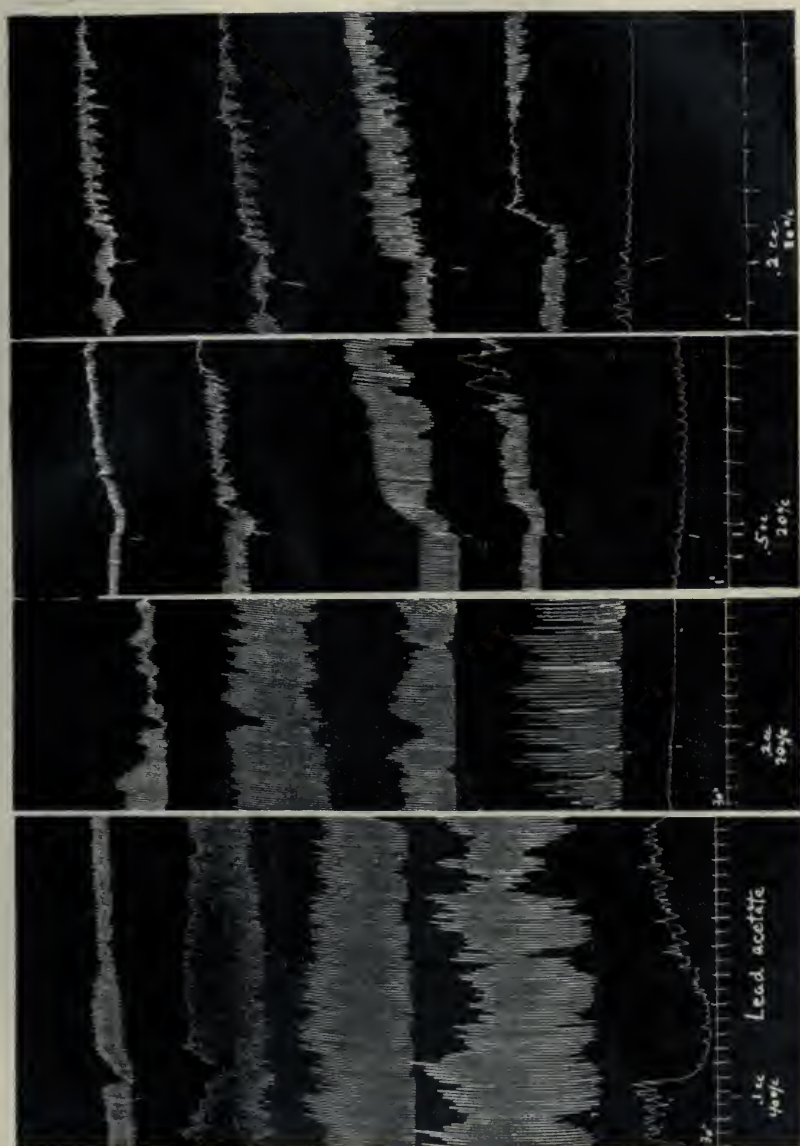


FIG. 4. DIFFERENCES IN THE ACTION OF LEAD ACETATE ON DIFFERENT PARTS OF THE BOWEL

With small doses the effects in small intestine and colon are similar. With larger doses the main effect in the small intestine is stimulation and in the colon inhibition.

Ammonia. "Stronger ammonia water" was used

	0.05 cc.	0.05 cc.	0.1 cc.	0.1 cc.
D.....	--+	0	+-	++
J.....	+-	+	-++	+++
M.....	---	--	0	+++
I.....	---	--	--	+---+
C.....	-++	+	0	-++

Apocodein hydrochlorid—1 per cent solution

	0.1 cc.	0.2 cc.	0.15 cc.	0.2 cc.	0.2 cc.	0.2 cc.	0.25 cc.	0.25 cc.	0.5 cc.	0.5 cc.	1 cc.	1 cc.	2 cc.
D....	0	+ Irreg.	+	++	+	+	+	0	+	++	+	Irreg.	--
J....	0	++	+	0	++	+	+	+-	+-	+	+-	++	---
M....	-?	+	++	+-	+	0	+	-	+	+	+-	+	---
I....	0	+	++	+	0	++	+	Irreg.	+	+	-	+	--
C....	+?	+	+	+	0	+	+	+	0	?	+-	+	--

Benzene. This substance gave peculiarly erratic results

	1 cc.	1 cc.	1 cc.	1 cc.	2 cc.
D.....	+	+--+	-+	+	+++
J.....	++	-++	++	--	-+
M.....	0	-++-+	0	-0	-
I.....	+	++	0	-	-
C.....	++--	--	-	-	---

Hydrogen peroxid—3 per cent solution

	0.1 cc.	0.2 cc.	0.2 cc.	1 cc.	1 cc.	2 cc.
D.....	+	0	+	-+	+++	-+-
J.....	+	0	0	-+-	++	-++-
M.....	?	+	+-	---	++	-+--
I.....	?	0	+-	+-	++	-+--
C.....	?	++?	-+	-+	-	+----

Lead acetate—20 per cent solution

	0.05 cc.	0.1 cc.	0.2 cc.	0.2 cc.	0.5 cc.	2 cc.	2 cc.	4 cc.	5 cc.
D.....	-	Irreg.	Irreg.	-	-++	++	++	+	--+++
J.....	-	0	-	-	-++	++	++	---++	--+++
M.....	-	0	-	0	++	-++	++	---++	--+++
I.....	0	0	0	0	-++	-+++	-+++	++	--+++
C.....	+?	0	--	--	-+	---	---	---	---

Lithium carbonate—0.5 per cent solution

	5. cc.	10 cc. ^a	10 cc.	12 cc.
D.....	+	?	—	+
J.....	++	—	++	+++
M.....	—	—	+	++
I.....	—	—	+	—
C.....	0	?	0	++

Nicotin—1 per cent solution

	0.1 cc.	0.1 cc.	0.1 cc.	0.1 cc.	0.1 cc.	0.15 cc.	0.2 cc.	0.25 cc.	0.3 cc.	0.3 cc.
D	+	+-+	+-	+-+	+	+	Irreg.	+-	++-	+++
J.	+	++	+-	+++	+	+	+ Irreg.	-+0	+-	+
M.	-+	—	+-	-+	+	+	-+0	-+0	++-	+-
I.	+	++++	—	++-	++	-+	++-	Irreg.	++-	++
C	---+	-+	++	+++	+++	---+	++	-+	---	++

Pituitrin—a commercial 1: 4000 solution was used

	0.05 cc	0.05 cc.	0.05 cc.	0.2 cc.	0.25 cc.	0.4 cc.
D.....	—	—	+	---+0	---+0	-0
J.....	—	++	0	+-	++	-0
M.....	—	-+0	0	-+0	-+0	—
I.....	—	+-	0	---+	+	—
C.....	—	—	—	---+	-+	++

Potassium iodid—25 per cent solution

	0.1 cc.	2 cc.	5 cc.	5 cc.	6 cc.	6 cc.	6 cc.
D.....	+	+-—0	++	+	+-	+-—	+++
J.....	+	+	++	+	+-	+-	+++
M.....	+	0	+	+	—	++-	+++
I.....	+++0	—	+++	+++	+-	++-	+++
C.....	0	+-	+++	++++	+	?	---+

Sodium bicarbonate—10 per cent solution

	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.
D.....	+	—	+	—+	+++
J.....	+++	—	++	?	+++
M.....	+	+++	++	?	++
I.....	+++	—	++	—	+
C.....	?	+-	+	—+	+-

Sodium chlorid—26 per cent solution

	2.5 cc.	5 cc.	7.5 cc.	7.5 cc.	7.5 cc.	12 cc.
D.....	+	+	++	?	-++++	++++
J.....	-	-	-++	-++	-++++	++++
M.....	0	?	-+	-++	++	++-+
I.....	?	?	+--	-++	++	++++
C.....	-?	-?	---	?	---	++++

Sodium citrate—50 per cent solution

	0.5 cc.	1 cc.	1 cc.	2 cc.	2.5 cc.	2.5 cc.	4 cc.
D.....	?	+	+	++	+	+	++
J.....	0	++	++	++--	++	+	++--
M.....	0	++	++	++-	++	+	++--
I.....	++++?	+	+	+++	---	+	++--+
C.....	0	+	--	---	---	-?	+---

Sodium phosphate (Na₂HPO₄)—10 per cent solution

	3 cc.	7 cc.	10 cc.	10 cc.	10 cc.	12.5 cc.	15 cc.	15 cc.
D.....	+	-?	+++	-	++	++	+++	+
J.....	+	+	++++	++	++	+++	++	+++
M.....	0	?	+++	++	+	++	+-	++
I.....	-?	?	+	++	+	+	+-	++
C.....	-?	?	+	-+?	+	?	?	--

Sodium potassium tartrate—50 per cent solution

	2 cc.	2 cc.	2 cc.	3 cc.	3 cc.	4 cc.	4 cc.	5 cc.
D.....	+	+	+	?	+	+	++-+	++-+
J.....	+	+	+-	+	+	+	++	+-+
M.....	+	Irreg.	+-	+	?	++-	+++	++-+
I.....	+	++	Irreg.	+	?	++-	++-	++-+
C.....	+	+++	+	+	+	++	+++	+++

Sodium salicylate—25 per cent solution

	2 cc.	4 cc.	6 cc.	8 cc.	10 cc.
D.....	+---		+++	---	+
J.....	+---	++	++-	++	+
M.....	++-	+-	++-	++	+0
I.....	+---	+-	++-	+++	+-
C.....	+++	+++	-++	++	+-

Strychnin sulfate—1 per cent solution

	0.4 cc.	0.5 cc.	0.5 cc.	0.5 cc.	0.5 cc.	1 cc.	1 cc.	2 cc.	3 cc.
D.....	?	+	+	+		++	+	—	—
J.....	+?	0	+	Irreg.	+	++	— Irreg.	—	—
M.....	+?	Irreg.	0	Irreg.	0	+	—	— Irreg.	++
I.....	+?	+	—	+	++	++	—	+	?
C.....	0	?	+?	+?	+?	+	0	+?	?

With the following four drugs, the main effect was a depressant one:

Antimonyl potassium tartrate—10 per cent solution

	3.5 cc.	3.5 cc.	3.8 cc.	4 cc.	4 cc.	7.5 cc.	8 cc.	12 cc.
D.....	—+	+	—+	---	---++	—+	—+---	—+
J.....	—+	---	---	—	---	---	---	---
M.....	---+++-	---	---	---	---	—++	---	---
I.....	—+-	—+	---	—++	—+-	—+	---	---
C.....	--	---+	—+	-----	---+	---+	--	--

Formaldehyd—3 per cent commercial solution

	0.05 cc.	0.1 cc.	0.1 cc.	0.1 cc.	0.15 cc.
D.....	?	---	---	---	---
J.....	—	—+	—	---+	---
M.....	—	---	---	---++	---
I.....	+?	---+	---	---++	---
C.....	0	+-----	---	---	---

Sodium nitrite—30 per cent solution

	0.5 cc.	1 cc.	1 cc.	1 cc.	1.5 cc.
D.....	?	—	—	—	---
J.....	—	---	—	—	+---
M.....	+?	—	---	—	—
I.....	+?	---	+?	—	0?
C.....	—?	---+	---	---	---

Sodium sulfate—30 per cent solution

	7 cc.	10 cc.	11.5 cc.	15 cc.	28 cc.	30 cc.	30 cc.	30 cc.	40 cc.
D....	0	+	+	0	?	—+++	—	—	—+—
J....	+—?	—+?	++	+	?	—++++	—	—	—
M....	+	+	+	+	+	—++++	—	—	—
I....	+	0	+	+	+	n. g.	—	—	—++—
C....	—	+	?	?	—	—	—	—	Irreg.

Mixed effects were obtained sometimes with the following twenty-four drugs. The main effect was a stimulant one with the first eight. They are:

Chloroform
Codein sulfate
Copper sulfate
Glucose

Mercuric chlorid
Morphin sulfate
Sodium fluorid

The main effect with the following sixteen drugs was a depressant one.

Acetone
Alcohol (ethyl)
Apomorphin
Calcium chlorid
Calcium lactate
Carbon dioxid
Digitalis
Hydrochloric acid

Oxalic acid
Phenyl hydrazin
Picric acid
Quinin bisulfate
Quinin hydrochlorid
Senna
Sodium potassium taurocholaite
Zinc sulfate

Sample protocols follow:

	CHLORO- FORM 4 CC.	CODEIN SULFATE 4 PER CENT 4 CC.	COPPER SULFATE 30 PER CENT 0.05 CC.	GLUCOSE 25 PER CENT 4 CC.	MERCURIC CHLORIDE 1.25 PER CENT 0.15 CC.	MORPHIN SUL- FATE 1 PER CENT 5 CC.	SODIUM FLU- ORID 0.4 PER CENT 5 CC.
D.....	+	+	++	+	+++	+	+—++
J.....	+	—	0	++	+++	—	++++—++
M.....	+	—	++	—	+	—	++—+++
I.....	++	++	++++	—	—	—	+—++
C.....	—	—	+++	+	+	+++ Ampl.	+++

	ACETONE 5 cc.	ALCOHOL 10 cc.	APOMORPHIN 0.06 GRAM	CALCIUM CHLORID 10 PER CENT 5 cc.	CALCIUM IAC- TATE 10 PER CENT 8.5 cc.	CARBON DIOXIDE	DIGITALIS, TINCT. 0.2 cc.	HYDROCHLORIC ACID N 0.6 cc.
D.....	+-	-+--	+-	--+	-	-++	---	-0
J.....	---	-+---	---	--++	---	---+	-	---
M.....	---	---+--	+0	---+	---+	---+	+	---
I.....	0	-+---	-	--++	---	---+	+	+++
C.....	---	-+---	0	+--	---	---	++	+++

	OXALIC ACID 1 PER CENT 2 cc.	PHENYL- HYDRAZIN 1 PER CENT 0.3 cc.	PICRIC ACID 1 PER CENT 2 cc.	QUININ BISULFATE 5 PER CENT 0.5 cc.	QUININ HYDRO- CHLORID 5 PER CENT 0.5 cc.	SENNA, FLUID EX- TRACT 2 cc.	SODIUM POTASSIUM TAURO- CHOLATE 10 PER CENT 5 cc.	ZINC-SUL- FATE 10 PER CENT 0.2 cc.
D.....	-+	0	--	--	--	-+0	?	+-
J.....	---	-	-+	-	---	+	--	+-
M.....	---	+	?	+	---	+	+-	-
I.....	+++	0	-+	Irreg. ++ Ampl.	++ Ampl.	+-	+-	---
C.....	--	---	?	?	+	---	+-	---

Drugs which showed graded effects from duodenum to colon

A number of substances stimulated or depressed one end of the bowel more than the other, while a few stimulated one end and actually depressed the other. If, as the writer believes, the downward progress of material through the digestive tract is dependent upon a gradient of muscular activity, drugs which tend to steepen that gradient might act as purgatives; and drugs which tend to reverse the gradient might act as emetics. Reversal could be brought about by drugs which depress the upper part of the tract more than the lower, by drugs which stimulate the lower part more than the upper, or by drugs which depress the upper part while stimulating the lower. This idea will be discussed more at length in a subsequent paper. The following two lists show drugs which might tend to steepen or reverse the gradient.

Drugs which might steepen the gradient.

<i>Markedly</i>	<i>Occasionally</i>
Adrenalin	Apocodein hydrochlorid
Aloin	Atropin sulfate
Carbon dioxid	Hydrogen peroxid
Cascara	Sodium bicarbonate
Magnesium chloride	Sodium chlorid
Magnesium sulfate	
Phenol	
Sodium citrate	
Sodium phosphate (Na_2HPO_4)	

Drugs which might reverse the gradient.

<i>Markedly</i>	<i>Occasionally</i>
Copper sulfate	Glycerin
Digitalis	Morphin sulfate
Ergot	Nicotin
β -Eucaïn	Zinc sulfate
Ipecac	
Potassium cyanid	
Potassium iodid	
Potassium permanganate	
Sodium potassium tartrate	
Sodium salicylate	

In the first list will be found a number of well-known laxatives and drugs such as adrenalin and atropin whose tendency to move the bowels is less generally known. Similarly, in the second list, besides the well-known emetics, there are such drugs as digitalis, nicotin, morphin, potassium iodid and sodium salicylate, all notorious for their tendency to upset the stomach.

Drugs whose action was more marked in the colon than in the small bowel

The depressant effects of alum, carbon dioxid, cascara, senna and sodium nitrite were usually more marked in the colon than in any part of the small bowel.

Mercuric chlorid generally stimulated the colon less than the small intestine. Sodium salicylate sometimes stimulated the colon more than it did the small intestine.

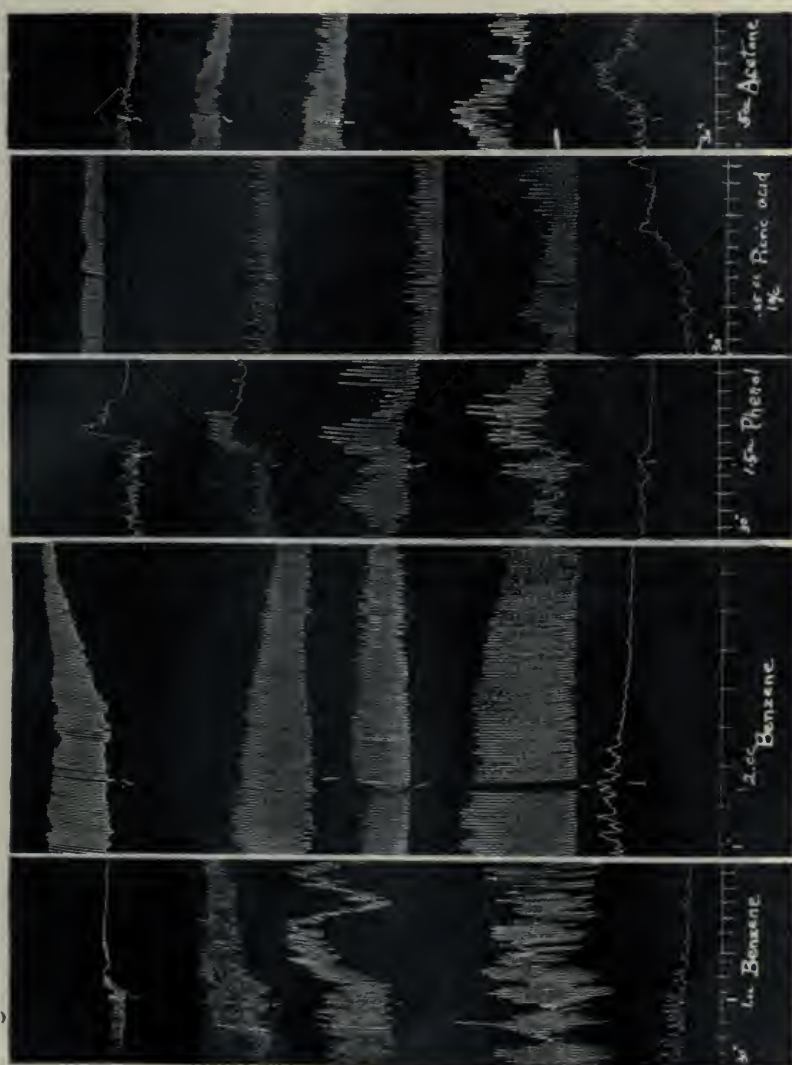


FIG. 5. DISSIMILAR EFFECTS IN SMALL INTESTINE AND COLON

Drugs whose action was different in small intestine and colon

Benzene usually stimulated the small intestine and depressed the colon. Similar effects were obtained with lead acetate and phenol. They were observed less constantly with chloroform, copper sulfate, sodium chlorid and sodium citrate. Morphin

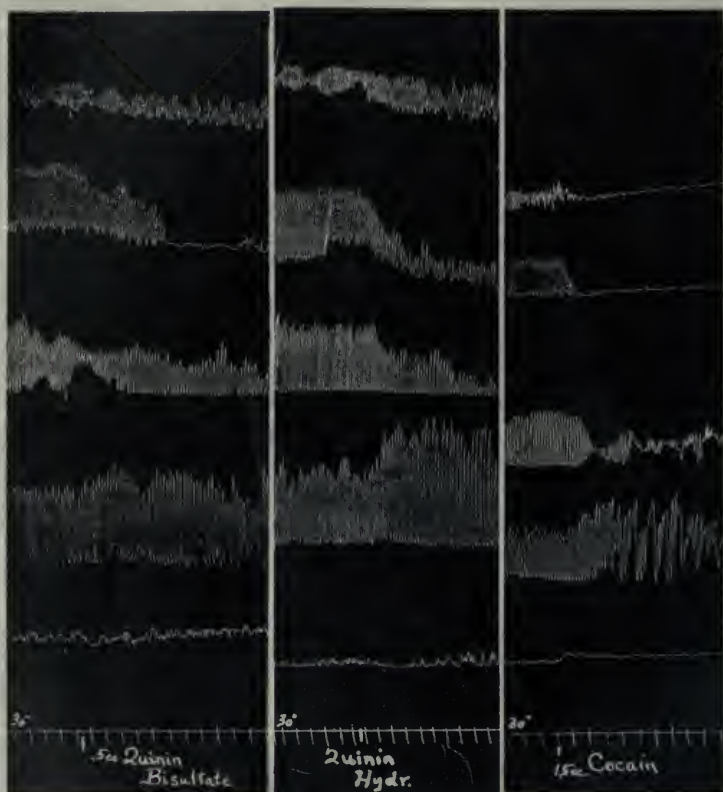


FIG. 6. DEPRESSION MOST PRONOUNCED IN THE JEJUNUM

The increase of amplitude in the ileum may be due to a loss in tone.

generally increased the amplitude of the colonic contractions while it sometimes depressed the activity of the small bowel. Picric acid, in several experiments, markedly stimulated the colon and at the same time, depressed the small bowel. Figure 5 shows some typical examples of these mixed effects.

Drugs which affected the jejunum particularly

There were a number of drugs which often depressed the tonus and rhythmicity of the jejunum to a marked degree. Figure 6 shows some typical examples. It is interesting to note that most of the following substances are nerve depressants.

<i>Marked effects</i>	<i>Occasional effects</i>
Chloretone	Acetone
Cocain hydrochlorid	Alum
Novocain	Anilin
Quinin hydrochlorid	Apomorphin
Quinin urea hydrochlorid	Chloral hydrate
	β -Eucain

Lithium carbonate and sodium phosphate sometimes stimulated the jejunum more than any other segment.

DOSAGE

For the convenience of other workers who may repeat some of the work I append the following list of concentrations of the various drugs; concentrations which give satisfactory reactions.

Acetone.....	1: 80
Adrenalin.....	1: 80,000,000
Alcohol (ethyl).....	1: 84
Aloin.....	1: 2,665
Alum $\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$	1: 4,000
Ammonia (strong).....	1: 8,000
Chloroform.....	Saturation
Anilin oil.....	1: 1,333
Antimonyl potassium tartrate.....	1: 950
Apocodein hydrochlorid.....	1: 800,000
Apomorphin.....	1: 400
Atropin sulfate.....	1: 800,000
Barium chlorid.....	1: 26,665
Benzene.....	1: 400
Bile (dog's).....	1: 80
Calcium chlorid.....	1: 2730
Calcium lactate.....	1: 500
Carbon dioxid.....	Saturation
Cascara, fluid extract.....	1: 265
Chloral hydrate.....	1: 400
Chloretone.....	1: 10,000

Cocain.....	1: 80,000
Codein sulfate.....	1: 4,000
Copper sulfate.....	1: 4,444
Digitalis, tincture.....	1: 200
Ergot, fluid extract.....	1: 200
Eserin.....	1: 80,000,000
Ether.....	Saturation
β -Eucaïn.....	1: 16,000
Formaldehyd.....	1: 4,000
Glycerin.....	1: 80
Glucose.....	1: 400
Hydrochloric acid n/1.....	1: 18,265
Hydrogen peroxid.....	1: 2,000
Ipecac, fluid extract.....	1: 320
Jalap, compound tincture.....	1: 200
Lead acetate.....	1: 500
Lithium carbonate.....	1: 8,000
Magnesium chlorid.....	1: 1,600
Magnesium sulfate.....	1: 1,665
Mercuric chlorid.....	1: 213,300
Morphin sulfate.....	1: 2,850
Nicotin.....	1: 800,000
Novocain.....	1: 160,000
Oxalic acid.....	1: 40,000
Phenol.....	1: 8,000
Phenyl hydrazin.....	1: 13,300
Picric acid.....	1: 26,600
Pilocarpin.....	1: 8,000,000
Pituitrin.....	1: 8,000,000
Potassium bromid.....	1: 400
Potassium chlorid.....	1: 330
Potassium cyanid.....	1: 800,000
Potassium hydrate.....	1: 100,000
Potassium iodide.....	1: 320
Potassium permanganate.....	1: 40,000
Quinin bisulfate.....	1: 16,000
Quinin hydrochlorid.....	1: 16,000
Quinin urea hydrochlorid.....	1: 12,000
Senna, fluid extract.....	1: 260
Sodium bicarbonate.....	1: 800
Sodium chlorid.....	1: 300
Sodium citrate.....	1: 800
Sodium fluorid.....	1: 5,000
Sodium hydrate n/1.....	1: 16,600
Sodium nitrite.....	1: 1,300
Sodium phosphate.....	1: 160
Sodium potassium tartrate.....	1: 400
Sodium potassium taurocholaite.....	1: 800

Sodium salicylate.....	1: 800
Sodium sulfate.....	1: 40
Strophanthin.....	1: 265,600
Strychnin sulfate	1: 80,000
Urea.....	1: 275
Urethane.....	1: 500
Zinc sulfate.....	1: 40,000

DISCUSSION

Although on the whole the different segments tend to respond in pretty much the same way to the various drugs, a number of striking differences have been observed. Some of these were very definite, and suggest marked differences in the metabolism of small intestine and colon. Others can be explained by differences in irritability and reaction time. Thus with some drugs the duodenum responded with a short drop followed by a permanent rise while the more sluggish colon showed only the rise or perhaps a drop so prolonged that one might think at first that the reactions in small intestine and colon were entirely dissimilar. Later, however, a rise might appear in the colon also.

Certainly it would seem advisable in all such pharmacologic studies henceforth to use four or five segments in the same beaker so that these local differences in action can be studied. Besides, the segments can serve as controls one upon the other. Every effort should be made to get healthy, well nourished rabbits for the work because even with those which appear to be normal, it is sometimes impossible to make certain segments contract regularly and with good amplitude. Such weakened segments will often respond abnormally to drugs or will greatly lessen the value of the records obtained. The threshold for drug action seemed to be about the same in the duodenum, jejunum, and ileum. It often seemed a little higher for the middle segment and perhaps still higher for the colon.

Some of the mixed effects obtained in the different segments seemed to be due to the fact that small doses stimulated and larger ones depressed. This was seen with chloral hydrate. If a segment happened to be less sensitive it might react as if to a smaller dose. Although it is stated in the literature that adre-

nalín stimulates the intestine in small doses I could not demonstrate it. Definite effects have been obtained with concentrations as low as 1:1,600,000,000 and they were always inhibitory in character.

The peculiar depression of the jejunum by a number of local anesthetics suggests the presence there of some special nerve endings. I have pointed out in a previous paper (16) that large fibers from the vagus nerve enter the small intestine at this point. It may be, however, that the jejunum sometimes loses so much tone simply because it has most to lose. As I have shown recently (17), when the segments are first put into the beaker the jejunum generally shortens most.

It must be remembered in all this work with excised segments that the results, although highly suggestive, cannot be taken over into practical pharmacology with full confidence until the experiments have been repeated on intact animals.

SUMMARY

Five segments excised from different parts of the rabbit's intestine have been studied under identical conditions in warm aerated Locke's solution. Seventy-six substances were tested. Some affected all the segments about equally, while others affected them unequally and even dissimilarly. A number produced effects more or less graded from one end of the tract to the other. It is suggestive that a number of the drugs which through their graded actions might tend to steepen the intestinal gradient are used as laxatives, and a number of those which might tend to reverse the gradient are well-known emetics.

Some of the drugs affected the colon particularly. Others had a peculiarly depressant effect on the jejunum.

A list of convenient dosages is appended.

REFERENCES

- (1) MAGNUS: Arch. f. d. gesammt. Physiol., 1908, cxxii, 210.
- (2) PANCOAST AND HOPKINS: Journ. Amer. Med. Assoc., 1915, lxxv, 2220.
- (3) SHAPIRO: Arch. f. d. gesammt. Physiol., 1913, cli, 65.
- (4) SICCARDI: Arch. p. l. Scienze Med., 1913, xxxvii, 58.

- (5) WASSERMANN: Arch. f. Exp. Path. u. Pharmakol., 1916, lxxix, 383.
- (6) SCHÜPBACH: Zentr. f. Physiol., 1907, xxi, 365.
- (7) ALVAREZ: Journ. Amer. Med. Assoc., 1915, lxxv, 391, notes 43 and 44.
- (8) ELLIOTT: Journ. Physiol., 1904, xxxi, 159; Ibid. 1905, xxxii, 416; Kuroda:
This Journal: 1916, ix, 187.
- (9) GASKELL: The Involuntary Nervous System, London, 1916, 45.
- (10) ELLIOTT: Journ. Physiol., 1905, xxxii, 423.
- (11) SALANT AND SCHWARTZ: Journ. Pharmacol. and Exper. Ther., 1917, ix, 497
and 511. Proc. Soc. f. Exp. Biol. and Med., 1916, xiv, 15.
- (12) SALANT AND MITCHELL: Amer. Journ. Physiol., 1916, xxxix, 355.
- (13) SALANT AND MITCHELL: Amer. Journ. Physiol., 1915, xxxix, 37.
- (14) ALVAREZ: Amer. Journ. Physiol., 1914, xxxv, 177.
- (15) ALVAREZ AND STARKWEATHER: Amer. Journ. Physiol., 1918, xlvi, 186.
- (16) ALVAREZ: Amer. Journ. Physiol., 1915, xxxvii, 276.
- (17) ALVAREZ: Amer. Jour. Physiol., 1918, xlv, 342.

THE LIBERATION OF THE INTERNAL SECRETION OF THE THYROID GLAND INTO THE BLOOD

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The object of this investigation was to determine whether it is possible to detect, in the blood coming from the thyroid gland, a substance whose physiological activity corresponds to that of the gland. There is at present no evidence which clearly shows the path by which the internal secretion of the thyroid gland enters the blood. Histological investigations of earlier workers led them to believe that the internal secretion reaches the blood through the thyroid lymphatics rather than directly through the blood capillaries, but this view has not been corroborated by more direct evidence.

Carlson and Woelfel (1) have attempted to detect the active substance of the thyroid gland in the lymph coming from the gland, but they did not succeed in obtaining good evidence of the presence of an active compound in the lymph which they collected. Owing to the very slow flow of lymph in the normal thyroid (about 2 to 5 cc. in twenty-four hours, in dogs) they were compelled to employ animals with goiterous glands in which the lymph flow from the thyroid is comparatively large. The lymph was collected through a cannula in the main neck lymph trunk below the point of entrance of the thyroid branches, after tying all of the branches above the thyroid. Chemical tests for iodine made with the thyroid lymphs of ten goiter dogs yielded negative results. Injection of the lymph into normal dogs yielded no definite evidence of the presence of active thyroid substance by the blood pressure or other reactions. Complete elimination of the thyroid and parathyroid lymph for thirty-six

to forty-eight hours in normal foxes did not induce symptoms of thyroidparathyroidectomy.

They also attempted to detect possible thyroid activity in the thyroid lymph by the acetonitrile test of Hunt (2, 3) but the results were again negative. As none of the reactions employed by these investigators was sufficiently sensitive or specific for thyroid, definite results could not have been obtained by them. It must be remembered, also, that the thyroid lymph was obtained from animals with goiters, and if the internal secretion of the thyroid enters the lymph, it is highly probable that lymph from a goiterous gland would differ materially from the lymph of a normal gland.

The results obtained in a number of investigations conducted in this laboratory with the "tadpole reaction" have indicated that this may be a sufficiently sensitive means of detecting such quantities of the internal secretion of the thyroid gland as might leave the gland in the blood. It has been shown by Gudernatsch (4) that when tadpoles are fed with thyroid gland they undergo marked changes manifested by rapid emaciation and acceleration of metamorphosis. Lenhart (5) found that this effect is proportional to the quantity fed and the amount of iodine present in the thyroid and that inorganic iodine does not produce the same effect. We have shown, further (6), that this effect is caused only by the iodine of the thyroid which is in specific combination.

When desiccated normal thyroid gland, containing a good store of colloid and iodine, is fed to tadpoles the emaciation and acceleration of metamorphosis occur so rapidly that it is impossible to observe which phenomenon precedes the other. If the dose is small or the iodine content of the gland is below that usually found in a normal gland, the first indication of activity is generally emaciation or retardation of growth and this is soon followed by angulation of the head. If the thyroid is very low in iodine, only the emaciation or retardation of growth may be observed for some time and finally the change in shape of the body and the appearance of the legs occurs somewhat sooner than in the control tadpoles. In our previous work it has been fre-

quently observed that when a specimen of thyroid that is very low in iodine is used or when a very minute dose of a more active thyroid is fed to tadpoles the principal indication of activity is emaciation or retardation of growth, the other changes occurring at about the same time as in the control tadpoles. It has been observed by Barfurth (7) that overfeeding with indifferent food causes metamorphosis to be postponed. This fact must not be overlooked, for when a preparation of thyroid with a very low iodine content is used a relatively large amount is generally offered to the tadpoles, and since the excessive amount of inert food is capable of postponing metamorphosis, the emaciation or retardation of growth is the only evidence of activity that might occur. Indeed, as already indicated, this is in accord with our experience. The reaction of tadpoles to the specific iodine compound of the thyroid is much more sensitive than the chemical tests at present available. In addition to the possibility of detecting the presence of small amounts of thyroid substance the quantitative character of the reaction renders it especially valuable as a means for determining the pharmacological activity of thyroid, by biological assay (8).

In this paper are reported the results of a preliminary set of experiments with blood obtained from the thyroid glands of three dogs, in which the tadpole reaction was employed. All of the blood specimens, after clotting, were dried at 55°C., and ground into a fine powder. The thyroid lobes of these animals were also dried and powdered, a small piece of each lobe having first been preserved for histological examination. A description of the procedures employed in obtaining the thyroid bloods is given in the following protocols.

Protocol—Dog 1

December 20, 1917. Adult male, weight 12.1 kgm.; thyroid lobes large and very vascular. Anesthetised with ether and dissected down, exposing both thyroid lobes. Isolated the right vago-sympathetic nerve in the neck. Inserted an oiled cannula into the vein at the upper pole of the right thyroid lobe and, without stimulation of the vago-sympathetic, collected thyroid blood (A); 136.5 grams of blood

was obtained in six and one-half minutes (21 grams per minute). As the blood in this cannula was clotting, another cannula was inserted into the vein at the lower pole of the same gland and the upper pole tied off. The right vago-sympathetic nerve was now ligated and cut and thyroid blood (B) was collected for two and one-half minutes during stimulation of the cephalic end of the vago-sympathetic nerve and for one-half minute after the stimulation was discontinued; 200 grams of blood was obtained in three minutes (66.7 grams per minute). The characteristic pallor of the gland due to vaso-constriction was not observed during the stimulation. The blood flow became greater after the upper pole was tied off. Now collected (through the same cannula) for five minutes without stimulation, specimen (C); 129.5 grams of blood was obtained (25.9 grams per minute). Clotting in the cannula occurred during the collection of this specimen. Finally obtained blood from the left iliac vein (D), and another specimen from the right thyroid artery (E).

The right thyroid lobe weighed 14.8 grams, the left lobe weighed 14.6 grams. A specimen of each lobe was saved for histological examination and the rest was desiccated and powdered, and preserved for feeding experiments and iodine determinations. No detectable iodine was found in these glands.

The blood specimens were allowed to clot and were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and saved for iodine determinations and feeding experiments. No detectable iodine was found in the amounts of blood available for testing.

On histological examination the thyroid lobes showed marked hyperplasia.

Protocol—Dog 2

January 8, 1918. Adult male; weight 10.87 kgm. Thyroid lobes small. Under ether anesthesia exposed both thyroid lobes; isolated the left vago-sympathetic nerve. Inserted an oiled cannula into the vein at the upper pole of the left thyroid lobe, clipped off the vein at the lower pole and collected through the cannula a specimen of thyroid blood (A): 152.0 grams of blood was obtained in three and one-half minutes (43.4 grams per minute). Now ligated and cut the left vago-sympathetic nerve and collected (through the same cannula) another specimen of thyroid blood (B): 69.5 grams of blood was obtained in 5 minutes (14.0 grams per minute). A clot formed in the cannula during the collection of the blood. The vein was tied off close to the cannula and an-

other cannula inserted into the same vein near the gland. During off and on stimulation of the cephalic end of the vago-sympathetic nerve collected specimen (C). The characteristic slowing of the blood flow and pallor of the gland was observed during each period of stimulation; 126 grams of blood was obtained in seven minutes (18.0 grams per minute). A specimen of ordinary venous blood was obtained from the right external jugular vein (D) and arterial blood from the femoral artery (E).

The thyroid lobes weighed 2 grams each. A specimen of each lobe was saved for histological examination and the rest was desiccated and powdered and preserved for the feeding experiments, and iodine determinations. No detectable iodine was found in these glands.

The blood specimens, after clotting were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and preserved for iodine determinations and feeding experiments. No detectable iodine was found in the quantities of blood available for testing.

Histological examination of the thyroid lobes revealed marked hyperplasia.

Protocol—Dog 3

February 12, 1918. Adult female; weight 9.125 kgm. Thyroid glands palpable. Anesthetised with ether and exposed both thyroid lobes; isolated the left vago-sympathetic nerve and separated the sympathetic from the vagus; attached guarded electrodes on the sympathetic nerve (without cutting the nerve). Inserted an oiled cannula into the vein at the upper pole of the left thyroid lobe and clipped off the vein at the lower pole. During off and on stimulation of the intact cervical sympathetic nerve, collected thyroid blood (A). The characteristic slowing of the blood flow was observed with each period of stimulation; 69.4 grams of blood was obtained in twenty minutes (3.46 grams per minute). Clotting in the cannula occurred and another cannula was inserted into the vein of the other lobe at the lower pole (the vein at the upper pole was not clipped off). A specimen of blood was collected without stimulation of the sympathetic (B): 10.1 grams of blood was obtained in four minutes (2.5 grams per minute). At the end of the collection of this specimen the blood in the cannula clotted; a cut was made in the vein near the cannula and blood allowed to run down alongside the cannula into a dish, after section of the vago-sympathetic nerve on this side (C): 33.8 grams of blood was obtained in ten minutes (3.4 grams per minute). In collecting the blood by this

means it was necessary to raise the gland and manipulate it frequently to facilitate the blood flow, thereby causing considerable massage of the gland during the collection of this specimen of blood. An indifferent blood specimen was obtained from the femoral artery (D).

The left thyroid lobe weighed 13.0 grams and contained 1.68 mgm. of iodine per gram of dried gland. The right lobe weighed 13.4 grams and contained 1.52 mgm. of iodine per gram of dried gland. A small piece of each gland was saved for histological examination and the rest was desiccated and powdered for the feeding experiments and iodine determinations.

The blood specimens, after clotting, were placed with the thyroid lobes into the drying oven at 55°C. When completely dried they were powdered and preserved for iodine determinations and feeding experiments. No detectable iodine was found in the quantities of specimens A and B available for testing; of specimen C 6 grams of the dried blood was available for iodine determination and in this specimen there was a detectable trace of iodine (somewhat less than 0.03 mgm. iodine in 6 grams of the dried blood).

Histological examination of the thyroid lobes showed the glands to be well stored with colloid material.

CHEMICAL TESTS FOR IODINE

After incinerating with sodium hydroxide, the different blood and thyroid gland specimens were tested for the presence of iodine by the method of Fresenius. In the quantities of blood that were available (0.4 to 18 grams) for testing, no detectable iodine was found in any of the bloods except specimen C of dog 3, which was collected during massage of the thyroid. This specimen gave a reaction equivalent to less than 0.005 mgm. of iodine per gram of dried blood.* The left lobe of dog 3 contained 1.68 mgm. of iodine per gram of dried gland and the right lobe contained 1.52 mgm. of iodine per gram of dried gland. The thyroid lobes of dogs 1 and 2 contained no detectable iodine.

* In another dog blood collected from the left lobe during sympathetic stimulation, with a flow of 1.43 gm. per minute, and another specimen collected from the right lobe during massage (after section of the right vago-sympathetic) with a flow of 19.4 gm. per minute gave negative iodine reactions. Tadpoles were not available. There was a large adenomatous tumor in the right lobe. The left lobe weighed 4.5 gm. and contained 1.81 mg. iodine per gram of dried gland. The right lobe weighed 7 gm. and contained 0.69 mg. iodine per gram of dried gland.

RESULTS OF FEEDING THYROID BLOOD TO TADPOLES

As in our previous work, the feeding was carried out in enamel-ware dishes each containing five tadpoles of uniform size in

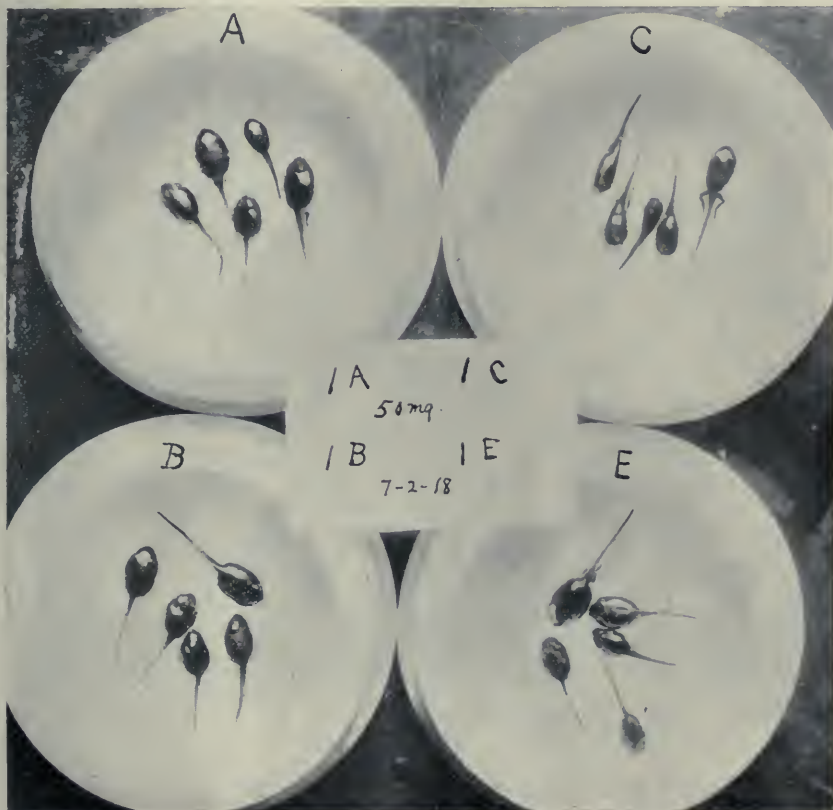


FIG. 1. BLOODS OF DOG 1 (SERIES I); FED IN DOSES OF 50 MGM. EVERY OTHER DAY FROM MAY 31 TO JULY 2

A, Thyroid blood, collected without stimulation of the vago-sympathetic; B, thyroid blood, collected during stimulation of the vago-sympathetic; C, thyroid blood, collected after cutting the vago-sympathetic; D, indifferent blood, obtained from the thyroid artery.

ordinary tap water. The substances to be tested were given every other day and fresh liver on the alternate days. The water was changed twice daily. The control tadpoles were given liver

only, every day. Two series were observed, the first series with tadpoles whose bodies were about 6 mm. in length and the second series with smaller tadpoles (about 3 to 4 mm. long). In the first series the desiccated bloods were offered in doses of 50, 100, and 200 mgm. and in the second series in doses of 75 and 100 mgm. The desiccated thyroid lobes were given in doses of 25 mgm. in both series. The feeding experiments were completed before the histological examination of the glands was made.

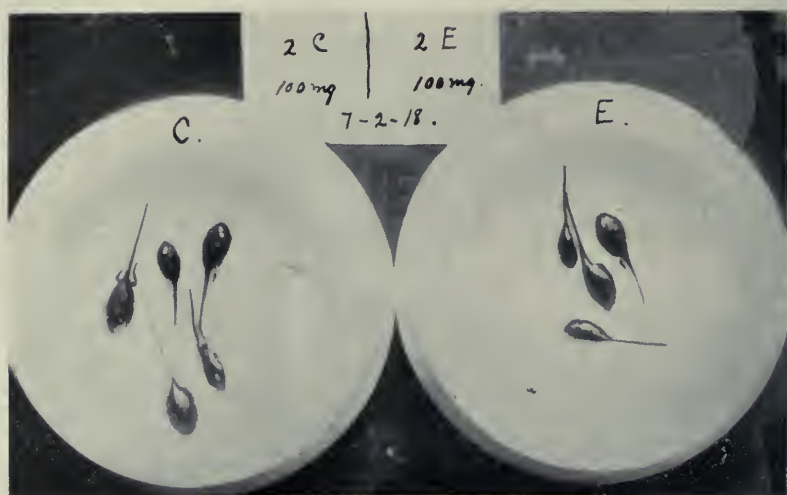


FIG. 2. BLOODS OF DOG 2, (SERIES 1); FED IN DOSES OF 100 MGM. EVERY OTHER DAY FROM MAY 31 TO JULY 2

C, Thyroid blood, collected during stimulation of the vago-sympathetic; *E*, indifferent blood, obtained from the femoral artery.

Although it seemed at first that all of the thyroid bloods caused some retardation of growth when compared with the effect of the ordinary (venous or arterial) blood, this effect was not sufficiently marked to be certain (figs. 1 and 2) except in the tadpoles getting the bloods from dog 3 collected during stimulation of the cervical sympathetic nerve, and during massage of the thyroid lobe (specimens A and C), which in both series caused definite uniform emaciation (figs. 3 and 4). Two important cor-

relative observations are associated with the activity of the bloods of dog 3, (1) the blood flow through the thyroid in this animal was considerably slower than in the other two dogs, and (2) the thyroid glands of dog 3 were normal and contained a good

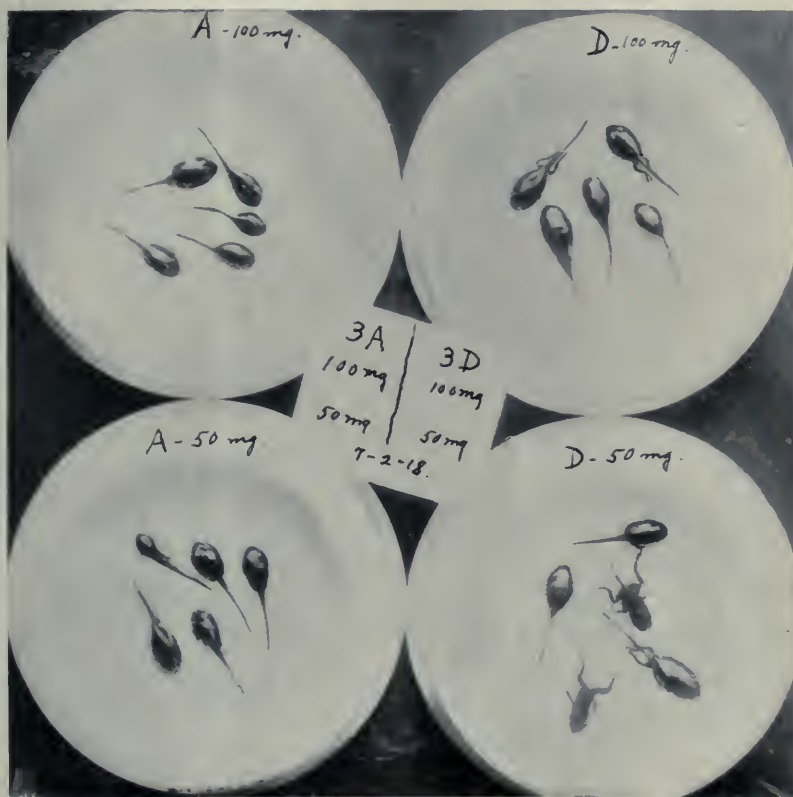


FIG. 3. BLOODS OF DOG 3, (SERIES I); FED IN DOSES OF 100 MGM. (UPPER SET) AND 50 MGM. (LOWER SET) EVERY OTHER DAY FROM MAY 31 TO JULY 2

A, Thyroid blood, obtained during stimulation of the cervical sympathetic nerve; D, indifferent blood, obtained from the femoral artery.

store of iodine, while the glands of the other two animals were hyperplastic and contained no detectable iodine. The activity of the blood obtained during stimulation of the sympathetic nerve is not necessarily due to the existence of secretory nerves

to the thyroid. For the slowing of the blood flow due to vasoconstriction when this nerve is stimulated may result in a higher concentration of the internal secretion of the thyroid in the blood coming from the gland if the rate of liberation remains steady.



FIG. 4. BLOODS OF DOG 3, (SERIES II); FED IN DOSES OF 75 MGM. EVERY OTHER DAY FROM JUNE 8 TO JULY 2

A, Thyroid blood, obtained during stimulation of the cervical sympathetic nerve; B, thyroid blood, obtained without stimulation of the sympathetic nerve; C, thyroid blood, obtained during massage of the gland; D, indifferent blood, obtained from the femoral artery.

The best example of such a condition is afforded by the adrenal gland in which it has been shown that the concentration of epinephrin in the blood coming from the gland, in general, varies inversely with the rate of blood flow through the gland (9).

The activity of the thyroid lobes corresponded with their histological appearance and their iodine contents. The lobes of dogs 1 and 2 which were hyperplastic and contained no detectable iodine caused practically no effect upon the tadpoles, while the lobes of dog 3 which were well stored with colloid material and



FIG. 5. THYROID LOBES OF DOGS 1, 2, AND 3 (SERIES II); FED IN DOSES OF 25 MGM. EVERY OTHER DAY FROM JUNE 8 TO JULY 2

had a good content of iodine caused very marked emaciation and augmented differentiation (fig. 5).

Figure 6 shows the control tadpoles for series 1 and 2. These tadpoles were given only liver (daily) and were used as controls for the tadpoles getting the indifferent bloods which were to be compared with those getting the thyroid blood.

All of the figures except figure 7 are reduced to $\frac{4}{5}$ of the actual size.

Figure 7 shows the histological structure of the thyroid glands of dogs 1, 2, and 3.



FIG. 6. CONTROLS. (SERIES I AND II); FED WITH FRESH LIVER ONLY, EVERY DAY

Series I, from May 31 to July 2; series II, from June 8 to July 2.

It is interesting to note that massage of the thyroid gland apparently is capable of liberating the active material from the gland into the blood. This also is in accord with what has been shown to occur in the adrenal under similar conditions (10, 11). It would not be profitable to speculate as to whether the histological condition of the gland or the slow blood flow resulting in

a high concentration of active material in the blood coming from the thyroid in dog 3 is the more important factor in the result obtained on tadpoles with specimen A. Concentrating the active material in the thyroid blood might be a means of obtaining quantitative information on the rate of liberation of the thyroid secretion. If the active material in the thyroid blood is the same as that in the gland, it is probable that concentration could be effected by the method of alkaline hydrolysis employed by Kendall (12). As tadpoles are available during a certain period of the year only, this and other suggestions have been reserved for future study.

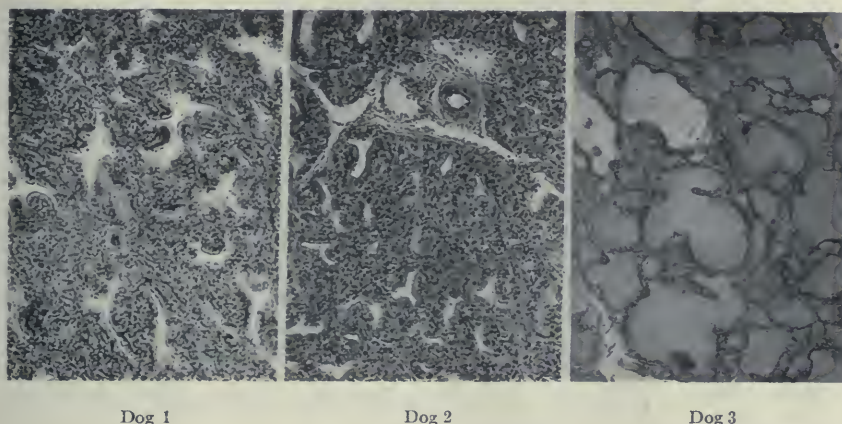


FIG. 7. PHOTOMICROGRAPH OF SECTIONS OF THYROID OF DOGS 1, 2, AND 3 ($\times 33$)

SUMMARY

1. An attempt was made to detect in the blood coming from the thyroid glands of three dogs, a physiologically active secretion, by feeding the dried blood to tadpoles.

2. One dog, whose thyroid glands were rich in colloid and had a good iodine content, yielded evidence of an active secretion into the blood collected from the glands during massage and during stimulation of the cervical sympathetic nerve. As indicated in the text this result yields no evidence of the existence of secretory nerves to the thyroid for it is not possible to know the rate of liberation of the secretion, and an increased concentration of

the secretion in the thyroid blood alone can not be taken as evidence of increased liberation.

3. Two dogs whose thyroid glands were hyperplastic and contained no detectable iodine yielded negative results.

REFERENCES

- (1) CARLSON, A. J. AND WOELFEL, A.: On the internal secretion of the thyroid gland. *Jour. Biol. Chem.*, 1909, vi, p. xv; *Amer. Jour. Physiol.*, 1910, xxvi, 32.
- (2) HUNT, REID: The effects of a restricted diet and of various diets upon the resistance of animals to certain poisons. *Hygiene Lab. Bull.* no. 69, U. S. Public Health and Marine Service, Washington, D. C.
- (3) HUNT, REID, AND SEIDELL, A.: Commercial thyroid preparations and suggestions as to the standardization of thyroid. *Jour. Am. Med. Assoc.*, 1908, li, 1385.
- (4) GUDERNATSCH, J. F.: Feeding experiments on tadpoles. I. The influence of specific organs given as food, on growth and differentiation. *Arch. f. Entwickl. Mechn. d. Organ.*, 1912-13, xxxv, 457. Feeding experiments on tadpoles. II. A further contribution to the knowledge of organs with internal secretions. *Am. Jour. Anat.*, 1913-14, xv, 431.
- (5) LENHART, C. H.: The influence upon tadpoles of feeding desiccated thyroid gland in variable amounts and of variable iodine contents. *Jour. Exp. Med.*, 1915, xxii, 739.
- (6) ROGOFF, J. M., AND MARINE, DAVID: Effect on tadpoles of feeding thyroid products obtained by alkaline hydrolysis. *Jour. Pharm. Exp. Ther.*, 1916, ix, 57.
- (7) BARFURTH, D.: Versuche über die Verwandlung der Froschlarven. *Arch. f. mikr. Anat.*, 1887, xxix, 1. Der Hunger als förderndes Prinzip in der Natur. *Arch. f. mikr. Anat.*, 1887, xxix, 28.
- (8) ROGOFF, J. M.: A method for the standardization of thyroid preparations. *Journ. Pharm. Exp. Ther.*, 1917, x, 199.
- (9) STEWART, G. N., AND ROGOFF, J. M.: The relation of the rate of the spontaneous liberation of epinephrin to the rate of blood flow through the adrenals. *Amer. Jour. Physiol.*, 1917, xlv, 149.
- (10) STEWART, G. N.: The alleged existence of adrenalin (epinephrin) in pathological sera. *Jour. Exp. Med.*, 1912, xv, 547.
- (11) STEWART, ROGOFF, AND GIBSON: The liberation of epinephrin from the adrenal glands by stimulation of the splanchnic nerves and by massage. *Jour. Pharm. Exp. Ther.*, 1916, viii, 205.
- (12) KENDALL, E. C.: A method for the decomposition of the proteins of the thyroid, with a description of certain constituents. *Jour. Biol. Chem.*, 1915, xx, 501.

NOTE ON THE PREPARATION OF A SOLUBLE CONCENTRATED PRODUCT OF THE THYROID GLAND

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During the course of the investigation reported in the preceding paper, the opportunity was available to test the effect on tadpoles of a product of thyroid which was obtained in an attempt to extract a concentrated water-soluble compound. The product "A" obtained by alkaline hydrolysis of normal thyroids of hogs, according to Kendall's method (1), was subjected to further hydrolysis in water acidified with hydrochloric acid. When the substance was completely digested the resulting solution was filtered through a Chamberland filter. To the clear filtrate was added hydrated aluminum silicate (Lloyd's Reagent) and the mixture thoroughly shaken and filtered through paper. The hydrated aluminum silicate on the filter was thoroughly washed with water until all of the acid was washed out. The adsorbed product was now separated by slowly percolating through the material on the filter a dilute solution of ammonia in water until the percolate came through entirely colorless. The ammoniacal solution was then heated on a water bath and the ammonia driven off with the aid of a current of air passed through the solution. The resulting aqueous solution was reddish brown and on evaporation yielded an amorphous powder. This powder contained 13.44 mgm. of iodine per gram of dry substance and the product "A" from which it was obtained contained 16 mgm. per gram. A small quantity of the product was available for feeding experiments with tadpoles. This product showed very nearly the same degree of activity as the product "A" from which it was obtained. Both products were given to the tadpoles in

doses of 0.5 and 1 mgm. every other day. They caused extreme emaciation and differentiation. In the tadpoles getting 1 mgm. doses the effect was produced so rapidly that very little difference could be observed in the activity of the two products. The tadpoles getting 0.5 mgm. doses indicated a slight difference in activity in favor of the product "A." No attempt was made to determine whether the slightly greater activity of the product

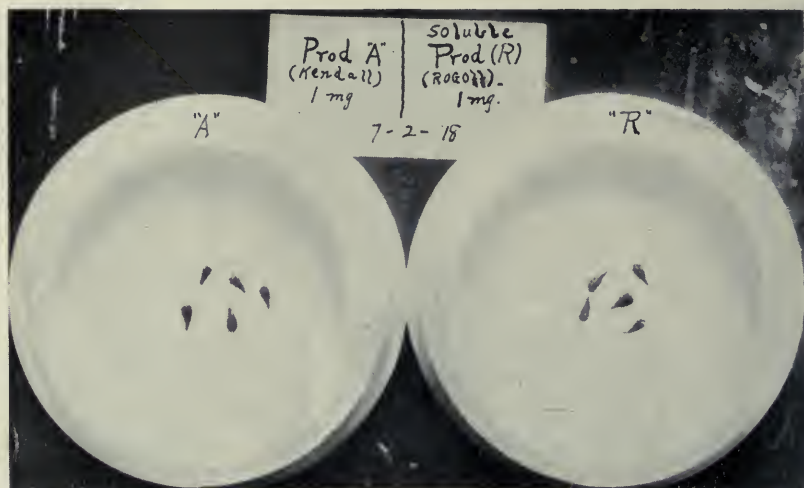


FIG. 1. PRODUCT "A" (KENDALL) (LEFT); SOLUBLE PRODUCT (ROGOFF) (RIGHT)

Fed in doses of 1 mgm. every other day from June 8 to July 2 (for controls, see figure 6, series II, in the preceding paper). Reduced to $\frac{1}{3}$.

"A" was proportional to the higher iodine content, as there was not sufficient material available for another series of experiments in which the effects of smaller doses could be observed and quantitative observations made.

Figure 1 shows the marked effect produced by both products when fed to tadpoles in doses of 1 mgm. every other day. The control tadpoles for this figure are shown in figure 6 (series II) in the preceding paper.

SUMMARY

The preparation of a concentrated active product of the thyroid gland, which is soluble in water, is briefly described.

REFERENCE

- (1) KENDALL, E. C.: A method for the decomposition of the proteins of the thyroid, with a description of certain constituents. *Jour. Biol. Chem.*, 1915, xx, 501.

THE APPLICATION OF A CONCENTRATED SOLUTION OF MAGNESIUM SULPHATE TO SCALDS AND BURNS

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I wish to place on record results of experiments with external application of a concentrated solution of magnesium sulphate carried out several years ago; the experiments were repeated since and similar results were obtained. My original experiments were briefly mentioned by Dr. Reid Hunt in a discussion of a paper of S. Solis-Cohen (1). At the period when the discussion took place we published numerous experiments on the action of magnesium sulphate on the nervous system; I abstained therefore from publishing my experiments on the local application of MgSO_4 on burns, etc., to avoid giving the impression that I consider magnesium sulphate as a panacea for all ills. We are engaged at present in experimental studies on the value of local application of a concentrated solution of magnesium sulphate to injuries caused by other procedures than scalding. I therefore decided to publish now my original experiments with hot water, presenting at the same time characteristic photographs illustrating the obtained results.

The experiments were made on non-injured and non-shaved rabbits' ears. The procedure was as follows. The animal was stretched out on a Cannon board with the head appropriately elevated so that glasses containing solutions could be put under the ears. The animal was deeply anesthetized, both ears were pulled down through the anterior opening of the board and about one-third of them was submerged for a short period in hot water. Then the water was removed and one ear was sub-

merged in a 25 per cent solution of epsom salt (1 mol. $\text{MgSO}_4 + 7$ mol. of water) and the other ear was submerged in a solution of NaCl (either 0.9 or 6 per cent). The ears were kept submerged in these solutions for about two or three hours, the solutions being changed two or three times during this period.

A number of experiments had to be made to previously establish the degree of temperature of the water and the length of time for the submersion of the ears in it which were necessary



FIG. 1

to bring out that degree of inflammation which could permit a doubtless conclusion as to the favorable action of the solution of magnesium salts. It was found that the inflammations brought about by water of a temperature less than $56^{\circ}\text{C}.$ were too insignificant to permit a statement as to the effect of MgSO_4 ; the difference between the two ears was too small and the recovery was fairly rapid and complete in both ears. A submersion in water of a temperature above $63^{\circ}\text{C}.$, for a three

minute duration or over, brought out such a degree of burn, with a subsequent destruction, of the submerged part of the ear, which did not permit the drawing of a definite conclusion as to the retarding effect of the submersion in magnesium sulphate solution. (It should be noted here that during the submersion of the ears the temperature of the water becomes fairly rapidly reduced).



FIG. 2

The experiments with submersion in water of temperatures lying between the two above mentioned degrees have always shown that the inflammation of the ear which was submerged in MgSO_4 was undoubtedly retarded or nearly completely prevented. However, for the purpose of obtaining photographic evidence of the usefulness of MgSO_4 the temperature of the

water had to be above 60°C. and the duration of the submersion in it could not exceed two minutes. The obtained results are well expressed by the reproduction of the following two figures.

Figure 1 is from a photograph taken about three hours after the ears were removed from both solutions in which they were submerged for two hours. The right ear was kept in a 25 per cent solution of MgSO_4 and the left ear in a solution of NaCl 0.9 per cent. They were submerged in these solutions immediately after the removal from the hot water. The latter had an original temperature of 62°C. and the ears were kept in the water for *two minutes*. The right ear (MgSO_4) was erect and appeared to be normal; the left ear (normal saline) was swollen, heavy, hot and red.

The ears of the rabbit in figure 2 were kept for *three minutes* in water of an original temperature of 62.5°C. The right ear was submerged in a 25 per cent solution of MgSO_4 and the left ear in a 6 per cent solution of NaCl , for three hours. The photograph was taken sixteen days later. Of the left (NaCl) ear about one-third sloughed away and the stump was healing. On the right ear there was some sloughing at the tip which was in process of healing.

I had occasions to see cases of burns in human beings. First and second degrees of burns are invariably arrested in their development when molecular solutions of MgSO_4 has been applied early. Third degrees of burns ran as a rule a more favorable course under application of MgSO_4 than under any other treatment. Higher concentrations than 25 per cent seem to exert a still better influence. The favorable action of the application of MgSO_4 in advanced stages of burns of second and third degree is less striking, especially on account of the infection present; but even in this stage it seems to exert a favorable influence and ought to be used either in combination, or alternately, with antiseptics.

REFERENCE

- (1) SOLIS-COHEN, S.: Jour. Amer. Med. Assoc., liii, p. 1892.

A TRANSPARENT CELLULOID RENAL ONCOMETER OR PLETYSMOGRAPH¹

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Visceral pletysmography or oncometry dates from the time of Roy (1) and of Cohnheim and Roy (2), who were the first to study the physiology of the visceral circulation by means of specially constructed pletysmographs or oncometers. Each instrument consisted of two appropriately shaped shells hinged together, the cavities connected with a common piston recorder. A piece of peritoneal membrane closed the hollow of each shell which in a moist condition was easily forced to conform to the contour of the enclosed organ by the hydrostatic pressure of the surrounding oily transmitting medium. Owing to the leaking of oil and the liability of pressure upon the hilus vessels, however, Schaefer and Moore (3) introduced their air transmission pletysmograph which incidentally proved to possess greater sensitiveness than the earlier oil method. Their instrument consisted of a rectangular gutta-percha box and a glass lid sealed together with vaseline, and connected either with a piston recorder or a tambour. For the intact intestine, Bayliss (4) employed a method similar in principle to that of Roy, which consisted in lightly pressing the intestine between a glass plate and a rubber membrane on the end of a thistle tube. Edmunds (5) described a liver pletysmograph which was made of two rubber bags held in

¹ Roy (1) states "For convenience of description I have been recommended to give a name to this form of instrument and will, therefore, refer to it under the name of 'Oncometer,' from the Greek word 'Onkos,' 'bulk.' " The terms "oncometer" and "pletysmograph," therefore, require a qualifying adjective denoting for what organ used, and may be used interchangeably.

place by leaden shells. His instrument possessed the advantages of air transmission, as well as leaving exposed no segment of the rubber bags.

Although there are some minor objections to the method of oncometry, its employment has been greatly restricted because of the lack of cheap, serviceable and durable instruments. An attempt to meet this situation is found in the employment of such materials as gutta-percha (3), dental composition (6), glass (7), lead (5), plaster of Paris (8), tin ointment boxes (7), aluminium soap boxes (9), brass (7), and celluloid soap boxes.

The present article deals with the use of celluloid which, in addition to meeting the requirements already mentioned, possesses the following qualifications: It makes a lighter or a less bulky instrument than glass, gutta-percha, plaster of Paris, and most of the metals. Its transparency permits a certain amount of observation, without removal of the instrument from the animal. Its strength and durability insure it against breakage through the rough handling of students. It is sufficiently rigid to withstand the fluctuations of intra-abdominal pressure, and yet flexible enough to allow the shells to fit into a self retaining dovetail joint. The exit tube can be made of the same material and is not detachable from its shell. Celluloid can be worked in the laboratory.

The molded celluloid forms possess the remote disadvantage of being non-resistant to very hot water and possibly the heat of direct sunlight. It is suggested that when not in use the shells be kept fitted together and bound lightly with rubber bands both crosswise and lengthwise. Although the process of manufacture is at first tedious, these serviceable instruments will amply repay anyone for his trouble.

METHOD OF PROCEDURE

The mold for pressing the shells is made of hard maple, as this wood withstands the heat well, and contains but a few pores in which the warmed celluloid can run. The female portion is made from an even surfaced block in which is cut an appropri-

ately shaped hole and hilus groove. The male part is made of a similar block, on which is mounted a half cylinder, rounded at the ends and a hemi-cylindrical ridge for the hilus groove. Allowance of one-eighth inch is made for the thickness of the celluloid. Oncometers with cavity lengths of 4, 4.5, 7 and 8 cm. were made, to accommodate kidneys of different sizes varying from that of a rabbit to that of a large dog.



FIG. 1. VIEW OF THE TWO SHELLS

Note the dovetail joint. The exit tube in this instrument is made of opaque celluloid.

Two rectangular pieces of transparent celluloid sheeting, $\frac{75}{1000}$ or $\frac{100}{1000}$ inch in thickness, are softened for thirty to forty-eight hours in 3 per cent camphorated 83 per cent alcohol. The mold is warmed to about 70°C., which temperature is sufficient to further soften the celluloid and at the same time will not cause blistering. The softened celluloid is dropped for a minute in warm 83 per cent alcohol, and then dried with a towel as quickly as possible. After powdering with talc, each piece is pressed slowly in the mold which is left clamped or weighted until cold. The shell after drying, shrinking, and warping for several days, is reshaped by pressing in the female portion of

the mold and any plane surface (such as the back side of the male portion).

The horizontal flanges of the shells are trimmed, leaving for the lower, $\frac{1}{4}$ inch and for the upper, $\frac{1}{8}$ inch. The edge of the upper flange is beveled. The surfaces of contact of the two shells are sandpapered until there is perfect contiguity. Next, a narrow softened strip of celluloid is cemented to the rim of the lower shell with amyl-acetate-celluloid lacquer. The shells are held together symmetrically while the strip is being cemented, the latter fitting the beveled edge of the upper flange snugly. After several minutes the shells are carefully separated, care being taken not to injure the newly applied rim, and any excess cement is then wiped from the joint. For about five minutes light pressure is applied to the lower shell inverted on a glass plate. The shells are then replaced, and the strip allowed to dry and contract while the former are in apposition. This insures a perfect fit, as well as a dovetail joint which permits the shells to fit with a "click."

A celluloid tube is cemented into a hole in the center of the upper shell which can be made with a file point. The oncometer is completed by rasping off the rough edges and giving the entire instrument several coats of lacquer to restore its luster and transparency.

EXPERIMENTAL

It is possible by suddenly squeezing in one's hands a properly sealed oncometer, or by compression in the cuff of a sphygmanometer to obtain evidence of its slight compressibility. Maximum rises of the tambour line of about 15 mm. by the hand squeezing method, and about 6 mm. with the sphygmanometer at 400 mm. Hg. pressure were obtained with two instruments. During animal experimentation, rises of 100 mm. of the tambour writing point were not at all rare, which indicated that the recording capacity of these tambours had not been taxed in the mechanical compression experiments.

The procedure of experimentation on the living animals was modified several times. It was found that ligating a kidney after

a control period, or ligating one after both the oncometers had been placed, offered no advantages over placing the control oncometer empty within the abdomen. In the latter method, however, it was necessary to protect the hilus from hernia of the intestines, by a cork or glass disc, or by placing the hilus directly against the abdominal wall. In most of the experiments rubber bands were used as a precautionary measure against dislodgment during the severer tests. The abdominal cavity was closed by three layers of sutures, the edges of the incision fitting snugly around the long celluloid exit tube.

Seven large and medium sized dogs were submitted to the following experimental tests; thoracic compression, vagus stimulation (intact and each cut end), tying the vagus, cutting vagus, infusion of Locke's solution, abdominal pressure (by weights or by hand), artificial respiration and respiratory stimulation by (a) allowing anaesthetic to wear off, (b) second stage of anaesthetic administration, (c) strychnine (not spasms), and (d) sciatic stimulation. In most of these tests no movement of the empty oncometer's tambour occurred. In none was it more than a millimeter, which by liberal calculation was not more than 3 to 5 per cent of the increment recorded by the other instrument.

Violent thoracic and cardiac massage was also tried. This failed to affect the empty oncometer three times, while in three other trials the rise of the control tambour was 2.5, 3 and 6 per cent, as calculated against the other instrument's fall. Abdominal punching, without regard for the situation of the oncometers, was also tried. The percentage rise of the empty instrument in six trials was from 2 to 20 per cent of the increment recorded by the other tambour. The results in strychnine spasms were from 12.5 to 20 per cent. Although other factors must be considered in the interpretation of plethysmographic data in such severe tests, the occurrence of a maximum error of 20 per cent is quite permissible, since the method itself gives at best only relative results.

Post mortem examination in some cases showed the collection of from 0.1 to 0.5 cc. of blood in the empty oncometer. Judging from these few experiments, it seems probable that the thin

consistency of the lanolin and the large size of the hilus were contributory causes. The gravitation of blood to the kidney situation furnishes a hydrostatic wedge, which undoubtedly is rather easily penetrable into the vulnerable hilus, in cases of increased intra-abdominal pressure, unless the lanolin has been reinforced with beeswax or cotton wool.

CONCLUSIONS

A new material, celluloid, has been suggested for making oncometers.

The celluloid kidney oncometer is practically not at all compressible under the conditions of ordinary physiological procedure. In strychnine tetanic convulsions the maximum error due to oncometer compressibility was 20 per cent.

The author desires to thank Drs. H. C. Jackson and E. M. Ewing, whose molds and gutta percha oncometers suggested some of the phases of this work.

REFERENCES

- (1) ROY, C. S.: *Jour. Physiol.*, 1880-82, iii, 203.
- (2) COHNHEIM, J., AND ROY, C. S.: *Virchows Arch. f. path. Anat.*, 1883, xcii, 424.
- (3) SCHAEFER, E. A., AND MOORE, B.: *Jour. Physiol.*, 1896, xx, 1.
- (4) BAYLISS, W. M.: *Jour. Physiol.*, 1893, xiv, 303.
- (5) EDMUNDS, C. W.: *Jour. Pharmacol. Exper. Therap.*, 1914-15, vi, 569.
- (6) STARLING, E. H.: *Principles of Human Physiology*. 2d ed., Philadelphia, 1915, Lea and Febiger, 993.
- (7) JACKSON, D. E.: *Experimental Pharmacology*. 1st ed., St. Louis, 1917, C. V. Mosby Co., 223, 163.
- (8) EDMUNDS, CHAS. W., AND CUSHNEY, A. R.: *Laboratory Guide in Experimental Pharmacology*. 1st ed., Ann Arbor, 1905, George Wahr, 223.
- (9) SOLLMAN, T. H.: *Laboratory Guide in Pharmacology*. 1st ed., 1917, Philadelphia, W. B. Saunders, 169.

AN EXPERIMENTAL INVESTIGATION OF THE CAUSE OF EARLY DEATH FROM ARSPHENAMINE, AND OF CERTAIN OTHER FEATURES OF THE PHARMACOLOGICAL ACTION OF THE SUBSTANCE

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Within recent years an extensive literature dealing with the newer organic arsenic compounds has appeared. With the great bulk of this we are not immediately concerned in the work which is herein reported, and which is entirely experimental in nature. There are, however, a number of excellent papers in the literature which deal more or less directly with certain features of the problem on which we have been working. In this field should be mentioned especially the work of Ehrlich and Berthelm, Auer, Alwens, Luithlen, Schlasberg, Fleig, Hoke and Rihl, Burzi, Joseph, Roth, Myers and DuMez, and Danysz.

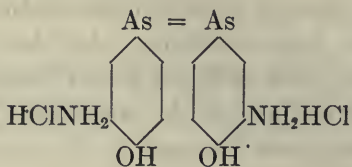
SCOPE OF THE PRESENT WORK

In the present experiments we have concerned ourselves mainly with the mechanism of acute death following the administration of arsphenamine. An analysis of the effects of various preparations of arsphenamine on the cardio-vascular apparatus was made with a view of discovering the cause of acute reactions, often seen in the clinic, from this drug. We have considered the possibility of precipitates forming in the blood stream, such as have been described by Joseph, Fleig, Danysz and others, with the possible consequence therefrom. We have also studied a number of intermediary and other products, incident to the manufacture of arsphenamine, as a possible cause for the acute

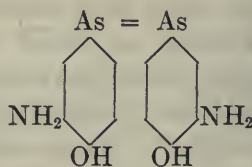
reactions. Finally, various remedies have been examined in the hope of finding some efficient method of treating acute collapse from arsphenamine.

Alkaline solutions of arsphenamine have been used in our experiments. It may be recalled that the neutral base of arsphenamine is insoluble in water. The acid salt (the dihydrochloride) is soluble in water. Addition of two molecules of sodium hydrate to one molecule of arsphenamine dihydrochloride forms the neutral insoluble base. Addition of a third molecule of sodium hydrate to the arsphenamine forms the monosodium salt which is soluble in water, while addition of a fourth molecule of sodium hydrate forms the disodium salt. Mixtures of mono- and disodium salts are obtained by adding appropriate quantities of sodium hydrate to the arsphenamine. Solutions of the monosodium salt are more readily precipitated by carbon dioxide, on exposure to the air, than are solutions of the disodium salt.

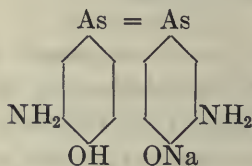
These reactions may be readily followed by reference to the following formulae:



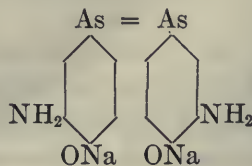
Dihydrochloride



Neutral base



Monosodium salt



Disodium salt

In making solutions of arsphenamine we have dissolved the weighed dihydrochloride in freshly glass-redistilled water and then added the theoretical amount of normal sodium hydrate to form the monosodium salt. We have generally employed 2 per cent solutions of arsphenamine, but in some cases 0.5 per

cent solutions have been used. In certain special instances, as will be pointed out later, solutions of arspenamine containing alkali in excess of the amount required to form the disodium salt have been used.

Dogs have been used in all of our experiments. They were usually anaesthetized with ether, although in a few experiments chloretone was employed as the anaesthetic.

ACTION ON THE CIRCULATION

When a slow injection of a dilute solution of arspenamine is made into an anaesthetized dog there are ordinarily produced no striking symptoms. If the dilution be 2 per cent or less and the rate of injection is such that not more than 10 mgm. per kilo of a good¹ preparation are injected over a period of several minutes, but little or no immediate change may be seen to occur in the cardio-vascular apparatus. This approximately corresponds to the dose and rate of injection usually employed in the clinic, although here solutions as dilute as 0.2 per cent or as concentrated as 2.5 per cent are employed by various clinicians. When, however, the rate of injection is accelerated and the dose is increased, definite effects soon begin to manifest themselves. Among the first of these is a slight but gradually progressive dilatation of the heart and a slow fall of blood-pressure. If the injection is made rapidly a rather marked, sudden fall of blood-pressure may occur, but the animal may partially recover from this effect in a few minutes after the injection is stopped. This fall in systemic pressure is apparently due largely to the alkalinity of the solution. Perhaps the endocardium is irritated and this results in a temporary weakening and dilatation of the heart; this effect is likely to be more marked following the first injection than after succeeding ones. Probably the right heart is more affected than the left, as may be expected from the pulmonary action to be described later.

¹ The toxicity of different preparations of arspenamine varies considerably in the dog. Further work along this line is in progress in this laboratory and we shall content ourselves for the present by referring to the various arspenamine preparations as "good" or "poor."

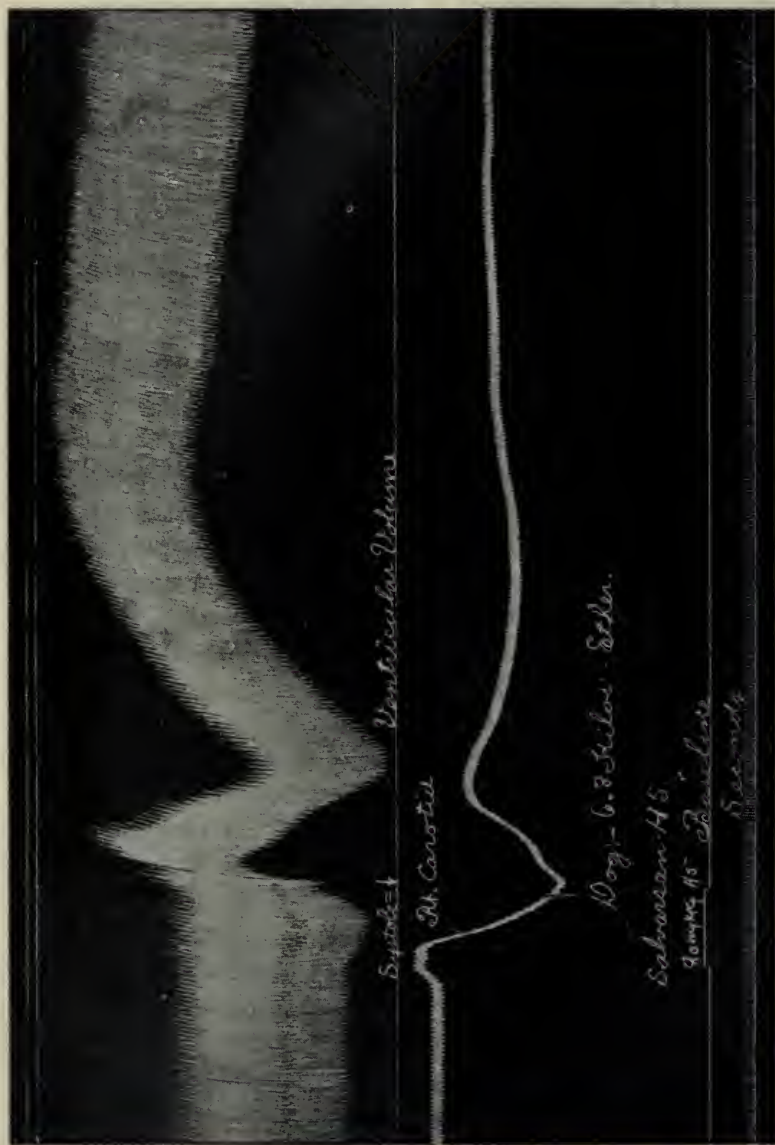


FIG. 1. CARDIOMETER AND BLOOD-PRESSURE TRACINGS FROM A DOG

Eighty milligrams of arsphenamine per kilo. of weight of the animal were injected in a period of about thirty seconds. This was a 2 per cent solution of the monosodium salt. There is a sudden and rather abrupt fall of the carotid pressure but in a short time this is mainly overcome and then a slower, gradual, secondary fall occurs. We believe the initial sudden fall is mainly due to the alkalinity of the solution; while the slower, secondary fall is apparently due mainly to the specific action of the arsphenamine itself. The marked dilatation of the heart is well shown by the rise in the cardiometer tracing. This dilatation is very persistent and is perhaps largely due to the rise in pulmonary pressure.



FIG. 2. TRACING SHOWING THE KIDNEY VOLUME, MYOCARDIUM AND RIGHT CAROTID BLOOD-PRESSURE IN A DOG OF 6.1 KILOS INJECTED WITH 25 CC. OF 2 PER CENT ARSPHENAMINE IN THE FORM OF THE MONOSODIUM SALT

The persistent shrinkage in kidney volume (probably partly, if not mainly, due to the lowered blood-pressure) and the steady, prolonged weakening of the heart with a gradual drop in systemic blood-pressure are shown. The dilatation of the heart is not so evident in this tracing as is the weakening of the organ.

These cardiac changes may be followed readily in the cardiometer and myocardiograph tracings. These observations fully corroborate the work of Auer and others.

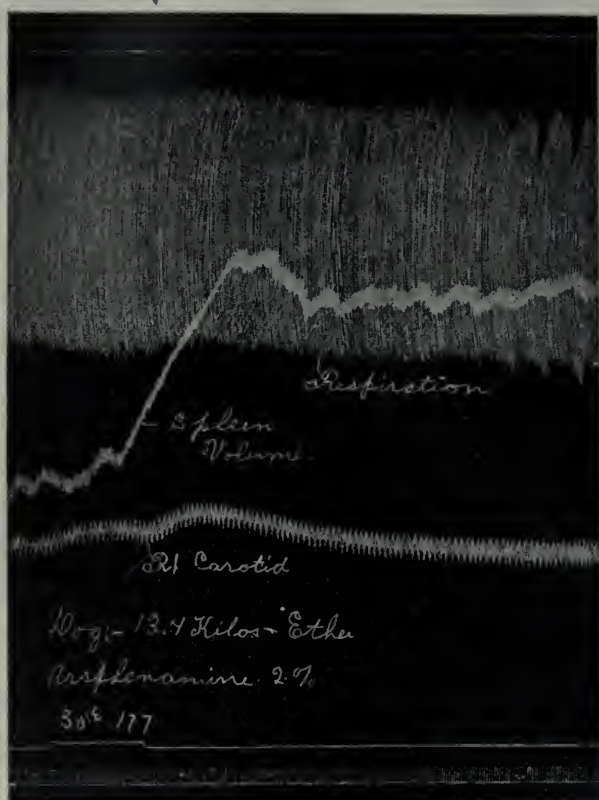


FIG. 3. TRACING SHOWING FROM ABOVE DOWN THE RESPIRATION, SPLEEN VOLUME AND CAROTID BLOOD-PRESSURE IN A DOG WHICH WAS INJECTED INTRAVENOUSLY WITH 30 CC. OF 2 PER CENT SOLUTION OF THE MONOSODIUM SALT OF ARSPHENAMINE

The dilatation of the spleen is well shown. At the same time there was a slight rise in blood-pressure.

The vascular reactions of the internal organs under the action of large doses of arsphenamine are interesting but difficult to explain. In a majority of cases we have seen a marked dilatation of the spleen occur as the drug was injected. This

appears to be, at least mainly, independent of the general blood pressure, and is perhaps due to a specific action on the arterioles of the spleen itself. On the other hand, the kidney usually contracts when a large dose is injected, and this contraction may sometimes be very marked indeed. The intestinal vessels apparently tend to dilate. The causes of these variable reactions are obscure. It is impracticable to make satisfactory perfusion experiments on these organs when excised, for the alkaline nature of the arspfenamine solution will probable cause contraction of the arterioles, independently of any specific effect which the arspfenamine might have. It seems probable that both central and peripheral influences are concerned in producing these results.

When a large dose of a rather toxic preparation is rapidly injected, a condition may be quickly produced in the animal which very closely resembles the condition described as shock, as defined by Mann, in which the blood-pressure is low, the respiration is feeble and slow or irregular, and the general vitality of the animal is so low that it cannot be revived, or at least only with great difficulty. It has been shown by Seelig and Joseph that in such conditions as this the vaso-motor center remains active. We have obtained oncometer tracings of the kidney, for example, which apparently illustrate this action of the vaso-motor center exercised over the internal organs, when collapse has been produced by the injection of toxic doses of arspfenamine, in a manner quite comparable to that shown in the classical experiment of Seelig and Joseph on the rabbit's ear.

The marked contraction of the kidney volume, which we have frequently seen following the injection of arspfenamine, may conceivably be related in some way with the production of nephritis. Several observers have noted that nephritis may be produced in experimental animals by arspfenamine. We have, however, no definite evidence of such intimate relation, and furthermore, in some animals it appears that the kidney volume may actually be somewhat increased by the injection of arspfenamine.

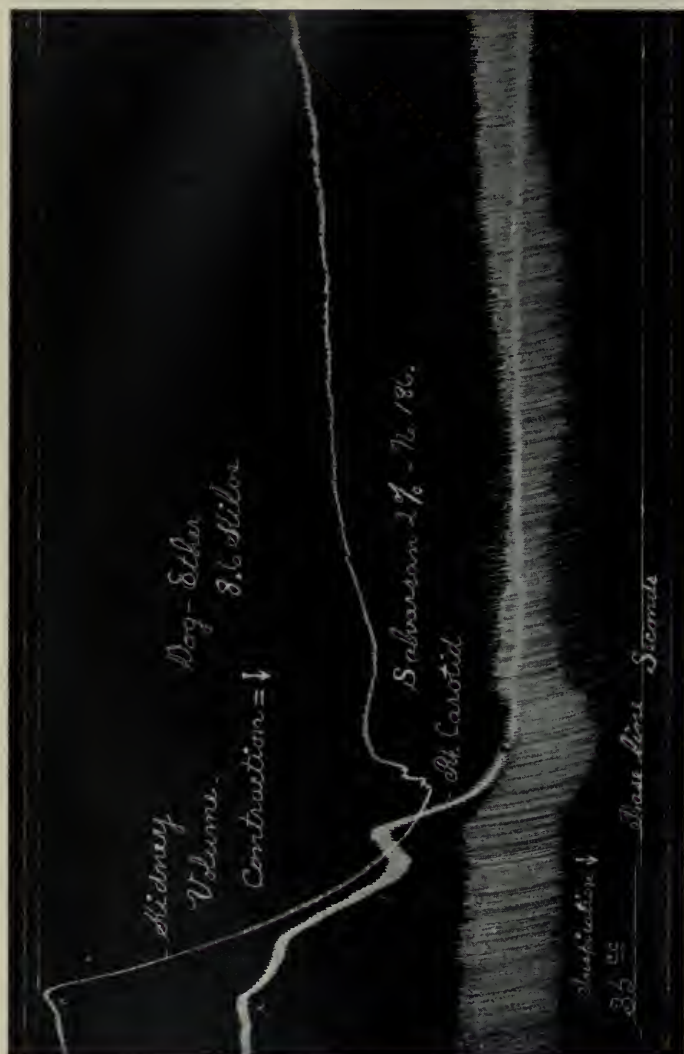


FIG. 4. TRACING SHOWING, FROM ABOVE DOWN, THE KIDNEY VOLUME, RIGHT CAROTID BLOOD-PRESSURE AND THE RESPIRATION IN A DOG INJECTED WITH 35 CC. OF 2 PER CENT MONOSODIUM ARSPHENAMINE

A profound and persistent contraction of the kidney volume follows the great fall in systemic blood-pressure. It is probable that the drug [here set up a condition in the animal closely resembling the state usually described under the term shock.

As pointed out earlier, one of the first phenomena noted when poisonous doses of arsphenamine are injected is a slight weakening, and especially a dilatation, of the heart. This action led us to seek for further disturbances in the circulation. We accordingly arranged to record the pulmonary blood-pressure from the left pulmonary artery by means of a mercury manometer. This method, of course, really records the pressure in the right pulmonary artery and right ventricle. The chest was opened widely and artificial respiration was given. Ether was generally used as the anaesthetic. Under these conditions it was found that large injections of arsphenamine solutions caused a very profound and progressively increasing rise in pulmonary blood-pressure. This frequently could be seen to begin before any appreciable change occurred in the carotid blood-pressure. It is well known that while the pulmonary blood-pressure is low, as compared with that in the systemic vessels, yet the pulmonary pressure, as measured by the mercury manometer, as a rule will change only a millimeter or two when changes of very considerable magnitude are being produced in the carotid artery, for example. For this reason a water manometer is often used to record the pulmonary blood-pressure. We have found, however, that the enormous changes in the lung circulation, under large doses of arsphenamine solution, can be very well shown with a mercury manometer. If a dose of 30 mgm. per kilo of a 2 per cent solution of arsphenamine in the form of the mono- or disodium salt be injected rapidly (within three or four minutes), the pulmonary blood-pressure may be increased by 100 per cent or more. At the same time a rather marked fall in systemic blood-pressure is likely to occur. These peculiar changes place the heart in a very unusual condition, for while the right side must do approximately 100 per cent more work than normally, at the same time the left side of the heart has from 25 to 50 per cent less work than normal to do. It was long ago noted by Auer that under arsphenamine the heart may pass into a rather unstable condition, from which it is liable to go into delirium cordis. During the course of our experiments we have seen delirium cordis develop and cause the death of the animal in a number of in-

stances. In these cases, however, the animal had received large doses of the drug and was already near complete exhaustion. It seems probable to us that the peculiarly trying and very unusual conditions under which the heart must work, when these enormous rises in pulmonary blood-pressure are produced by large doses of the drug, might very well so strain the heart that the development of delirium cordis might well be expected.

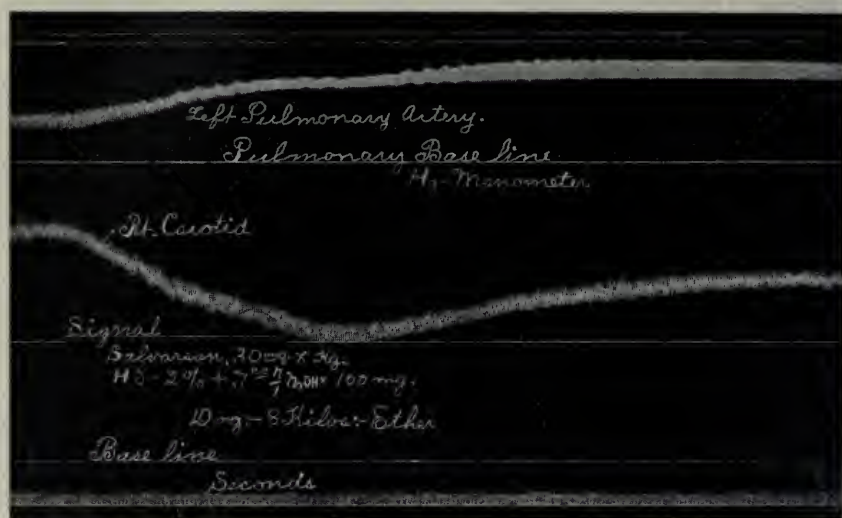


FIG. 5. LEFT PULMONARY BLOOD-PRESSURE (ABOVE) AND RIGHT CAROTID TRACING (BELOW)

Thirty milligrams of 2 per cent arsphenamine per kilo of body weight were injected into the femoral vein. The gradual; progressive rise in pulmonary blood-pressure is very evident. This high tension persists in spite of the later tendency of the carotid pressure to rise slightly, after the primary fall. It is seen that the pulmonary pressure remains at practically twice its normal height throughout the greater part of the tracing.

The cause of this increase in pulmonary pressure is at once a matter of interest. The possibility that it might be due to the formation of minute emboli from precipitation of the arsphenamine occurred to us early. It is conceivable that these might form and quickly lodge in the lungs and thus block to a marked degree the passage of blood from the right ventricle over to the left auricle. We have not been able to prove that this may not

occur, at least to some extent. We, however, do not believe that this is the chief cause, even if it contributes to the result in any degree whatever. For it can easily be shown that a dilute solution of sodium hydrate alone, of such approximate strength as to correspond to the amount of alkali used to make the monosodium salt of arsphenamine, will of itself, when given in good sized doses cause a very marked rise in pulmonary blood-pressure. We believe, therefore, that the alkalinity of the arsphenamine solution alone may be responsible for a considerable degree of rise in pulmonary blood-pressure. We, however, believe that the specific action of the arsenic compound itself is responsible for a part, and possibly for most, of the increase in pulmonary arterial tension. And it is our opinion that the rise in pulmonary arterial pressure is due to a direct stimulation and contraction of the muscle fibers of the pulmonary arteriole walls.

We wish to emphasize that we have seen this rise in pulmonary pressure only when large doses of the drug were injected. These have usually been in considerable excess over those generally used clinically. On the other hand, the mercury manometer which we have used to record the pulmonary pressure is relatively a very insensitive instrument, and with a water manometer, for example, marked changes in the pulmonary pressure would almost certainly have been shown by doses of the drug which were too small to show appreciable changes with the mercury manometer.

While large doses of concentrated (1 or 2 per cent) alkaline solutions of arsphenamine rapidly injected have always produced great rises in the pulmonary blood-pressure in our experiments, we can state that the slow injection of dilute (0.5 per cent) solutions of the drug may be successfully carried out without the production of any visible rise in pulmonary pressure, as observed grossly in the mercury manometer record.

It is not probable that a mere fall in the general systemic pressure is a sufficient reason to account for a marked, prolonged increase in pulmonary pressure. It is conceivable that the mere shifting of the blood around through the arterioles

on to the venous side of the circulation might be a sufficient cause to produce this pulmonary pressure rise. But we have seen the pulmonary pressure start to go up several seconds before any change whatever could be seen in the height of the systemic (carotid) pressure. And further, even though the carotid pressure may fall at about the same time as the pulmonary rises, yet we have seen the carotid pressure again go up to its previous level while the pulmonary pressure remained permanently high.

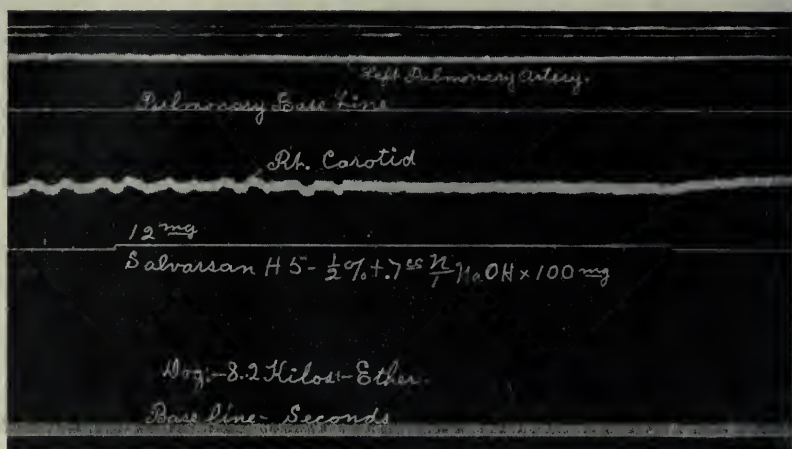


FIG. 6. PULMONARY BLOOD-PRESSURE (ABOVE) AND RIGHT CAROTID TRACING (BELOW)

Twelve milligrams of 0.5 per cent alkaline (monosodium salt) arsphenamine per kilo of body weight were injected intravenously, very slowly and over a prolonged period of time. Under these conditions of administration it is seen that but slight change occurs either in the pulmonary or the carotid blood-pressure. This action perhaps approaches very closely that which should be obtained clinically.

In the cardiometer tracings a dilatation of the heart is seen as one of the earliest cardiac actions of the drug. We suspect that the rise in pulmonary pressure quickly manifests itself in the right ventricle, and that dilatation of this chamber is chiefly responsible for the initial increase in cardiac volume as shown in the cardiometer tracing. A later weakening of the whole musculature of the heart may, of course, involve the left ventricle as well as the right.

Faintness, dyspnoea and circulatory disturbances are among the most common phenomena complained of in those patients who rapidly develop toxic symptoms when arspenamine is administered to them. We are of the opinion that in these cases a disturbance in the pulmonary circulation may be one of the chief factors concerned in the development of the symptoms.

It has been suggested that solutions of the disodium salt of arspenamine, or even solutions of the disodium salt containing a slight excess of alkali, might be less toxic than simple solutions of the monosodium salt. It has been our observation that the more alkali the solution contains, the greater has been the rise produced in the pulmonary pressure.

ACTION ON THE RESPIRATION

Moderate doses of a good arspenamine preparation injected slowly into an animal produce but little effect on the respiration. As the action of a large dose of arspenamine develops, however, the respiration gradually becomes slower and more shallow. The character of the respirations also progressively changes, so that there is a gradual transition from the mainly abdominal type of breathing to a form in which the intercostal muscles are mainly involved. In the final stages the respiration becomes slow, feeble and almost wholly of the thoracic type. It appears that this respiratory embarrassment results both from depression of the respiratory center and from the greatly depressed circulation; for artificial respiration is of no avail. It should be added that the respiration is not materially affected until late in the course of intoxication, when the circulation is greatly depressed and is beyond the stage where circulatory stimulants can be of any help.

Respiratory failure is probably the immediate cause of death in most instances of experimental arspenamine intoxication, as the heart may usually be seen to beat a short while after the respiration ceases. Frequently both the heart and respiration may be seen to stop simultaneously, especially if a rapid injection of a toxic preparation be given. These phenomena remind one of the cause of death under varying concentrations of chloroform vapor.

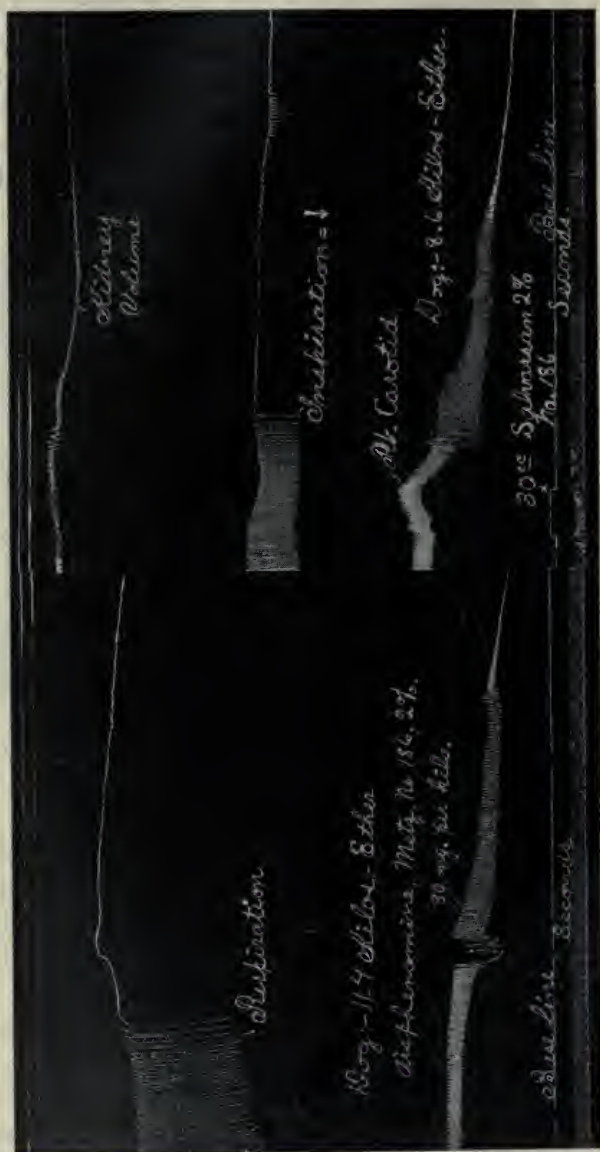


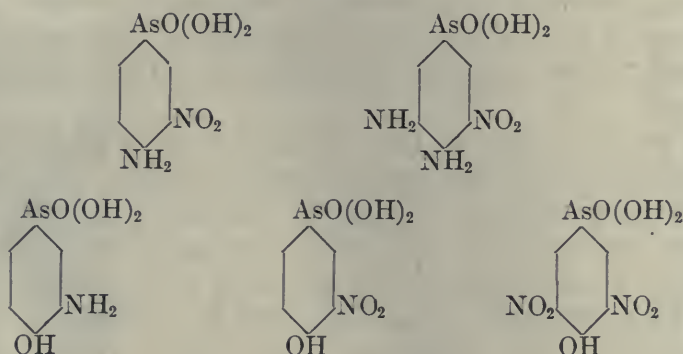
FIG. 7. DEATH RECORDS (MOUNTED TOGETHER) FROM TWO DOGS

The left hand tracing shows the respiration and the carotid blood-pressure. The right hand tracing shows from above down, the kidney volume, respiration and carotid pressure. These records show that the immediate cause of death under arsphenamine is respiratory paralysis. See text for full discussion.

THE RELATION OF INTERMEDIARY AND OTHER PRODUCTS OF ARSPHENAMINE TO THE TOXICITY THEREOF

It is definitely recognized now that different arspenamine preparations vary widely in their toxicity in animals. The reasons for such variation are obscure. We have attempted to throw some light on this matter by a study of certain intermediary and oxidation products which are formed in the process of manufacture of arspenamine, and which might conceivably be partly carried over into the final product.

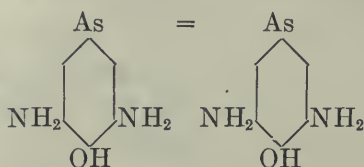
Through the kindness of Dr. C. N. Myers, of the Hygienic Laboratory, we have been able to examine the effects on the circulation of the compounds whose formulae appear below:



Solutions of these compounds were made, in every respect similar to those of arspenamine, and injected intravenously into dogs. Blood-pressure and myocardiographic or respiratory tracings were made in each case. None of these compounds, even if given in such immense doses as 500 mgm. per kilo of animal weight, produced circulatory effects in any way comparable with those following arspenamine. The lesser toxicity of these compounds may perhaps be accounted for by the arsenic being in the pentavalent form, unlike the arsenic in the arspenamine molecule which is trivalent. All of these intermediary bodies which contain nitro groups produced a deep yellow discoloration of the skin, subcutaneous tissues, mucous membranes, etc., connective and alveolar tissues being mostly affected.

No methaemoglobin could be detected in the blood under the conditions of our experiments.

Another body closely allied to arspenamine, and a possible by-product in the chemical manufacture of the latter, having four amino groups as shown in the formula,



was examined. A dog received 70 mgm. per kilo of body weight of 1 per cent solution of this tetramino compound without showing any marked, permanent *circulatory* depression. A similar dose of even a first class preparation of arspenamine will produce, at least, some lasting depression of the systemic blood-pressure.

In the last stage in the manufacture of arspenamine the precipitated base is repeatedly washed with ether. We examined the circulatory effects of such ether washings from three different lots of arspenamine. The ether washings were evaporated in vacuo to a small residue which was taken up with distilled water. The hydrochloric acid was then carefully neutralized with sodium hydrate and the solution was injected intravenously into the animal. As thus tested, these washings proved to be practically entirely inert. It is clear, therefore, that none of the foregoing compounds can play a significant rôle in the acute toxic action of arspenamine.

CONCERNING ARSPHENAMINE PRECIPITATES FORMING IN THE BLOOD STREAM

It was long ago shown by Joseph that acid solutions of arspenamine injected intravenously in concentrations above 0.1 per cent would produce precipitates in the blood, with consequent formation of emboli which tended to lodge in the lungs, right heart, etc. More recently Danysz, and perhaps others, have believed that arspenamine in alkaline solutions may undergo

precipitation in the blood stream and thus form insoluble masses which tend to lodge in the finer blood vessels, especially of the lungs, and thus cause grave symptoms. Danysz believes that, later, these insoluble masses again undergo solution, due to the solvent action of the blood and tissue juices, which, by combination with the precipitated drug masses, form complex, soluble, organic compounds which are forthwith carried onward in the blood stream and distributed to the tissues of the body. The chemical reactions of aqueous solutions of arspenamine in vitro seem to furnish evidence that this precipitation of the drug actually occurs in the living animal, for it can easily be shown that carbon dioxide, calcium salts, and phosphates, for example, cause precipitation of arspenamine from its alkaline solution in water.

This explanation of the toxic action of arspenamine does not seem to us to be particularly satisfactory. For it does not explain why different preparations should possess such varying degrees of toxicity. Furthermore, it is hardly probable that the composition of the blood varies so widely in different individuals as to cause precipitation with resultant "nitritoid" reactions and "anaphylactoid" symptoms in one individual, and no effects whatever in others treated with the same preparation of arspenamine. And, experimentally, we have seen no symptoms produced in our animals which seemed to us to demonstrate that, with alkaline solutions, precipitation and embolism occurred. Nor have we found emboli to be present in the lung vessels after injection of alkaline solutions; but we wish to emphasize that in this case we have made only gross examinations, and we cannot therefore be sure that microscopical examination might not show the presence of emboli in the finer arterioles.

We have, therefore, sought some further evidence on this subject of intravascular precipitation. Since carbon dioxide is one of the normal constituents of the blood, and is also one of the arspenamine precipitants, we have attempted to learn what effect this gas might have on the sudden development of severe symptoms from arspenamine, when the animal was made to

breathe for considerable periods of time into a closed bag from which the carbon dioxide could not escape but was continually rebreathed over and over.² Under these conditions we have found that the presence of large quantities of carbon dioxide in the respired air make no difference whatever in the toxicity of the arsphenamine injected, so long as sufficient oxygen is given to the animal.³ An 8 or 10 kilo dog may breathe for ten or twenty minutes to and from the bag, and the only change seen under progressively increasing dosage of arsphenamine injected intravenously is that the blood-pressure of the animal may be kept somewhat higher than ordinarily would be the case, because the asphyxia tends to stimulate the vasomotor center, and the respiration tends to show some embarrassment when the carbon dioxide content of the bag becomes very high. These are entirely normal phenomena under the conditions of the experiment, and both disappear at once when the animal is allowed to breathe fresh air. So far as we are able to determine, the forced rebreathing of carbon dioxide in this manner in nowise increases the toxicity of arsphenamine in anaesthetized dogs.

In other animals we injected calcium lactate (10 cc. of a 4 per cent solution) in small repeated doses and followed this with arsphenamine. Apparently this in no way rendered the drug more toxic. Calcium hydrate, likewise, did not alter the susceptibility of the animal to arsphenamine.

In a similar manner we injected a solution of sodium-dihydrogen phosphate into an animal, up to the limit of tolerance, without producing too great a change in the circulation and respiration. This was followed by an injection of arsphenamine. Apparently the toxicity of the drug is not noticeably influenced by any of these procedures.

² For this purpose we have used an ordinary rubber bag holding about three litres.

³ The oxygen was administered from a tank from which a tube led into the rebreathing bag.

SUGGESTION AS TO THE TREATMENT OF ACUTE ARSPHENAMINE
COLLAPSE

From time to time various drugs or other measures have been suggested to prevent the development of untoward symptoms, or to combat these if they should develop during or after the administration of the drug. Epinephrine has generally been first considered among these agents.

Our experimental observations in the use of this drug as an antidote for acute arspfenamine poisoning have been not at all promising. We have only a single suggestion to make in the line of treatment of those cases in which severe symptoms suddenly manifest themselves, either while the drug is being injected, or within a short period thereafter, and this consists in the administration of tyramine (parahydroxyphenylethylamine). This drug may be injected intravenously in cases of emergency, or it may be given intramuscularly or subcutaneously. We have undoubtedly prolonged the lives of animals severely poisoned by arspfenamine, when doses of 10 mgm. of tyramine were injected intravenously into animals weighing from 5 to 10 kilos. Tyramine stimulates the heart and produces a prolonged, sustained rise in systemic blood-pressure. When given intravenously it may also cause a rise in pulmonary pressure, even if this is already considerably raised by arspfenamine. Probably this stimulating action on the heart and arterioles by which the circulation is improved is the only way in which tyramine may be of any help at all to a patient in collapse. In dogs we have seen this stimulating action persist for over half an hour when the drug is given in a large dose intravenously. The effects progressively decrease after the initial rise in pressure, which requires from one to five or more minutes for its full development. The systemic pressure does not rise so high and the danger of acute dilatation of the heart is not so great as with the intravenous injection of epinephrine, but the effects of tyramine are much more lasting.

While we are by no means sure that the use of this drug might in any degree decrease the number of fatalities that from

time to time occur under the use of arsphenamine, yet we believe it is more logical, and more likely to be of real value in these cases than is any other drug with which we are acquainted. While a number of investigators have used tyramine clinically, Hewlett in particular has injected it hypodermically in doses of 40, 60, or 80 mgm. (average dose 60) and has, in general, found it to be satisfactory as a blood-pressure raising substance. Since tyramine stimulates the right ventricle along with the rest of the heart it may very well be considered to improve the circulation indirectly also, by thus helping to overcome the obstruction set up in the lungs as manifested by the increase in pulmonary blood-pressure.

We should mention that tyramine might, under certain obscure conditions, initiate cardiac irregularities; and in very rare instances delirium cordis might be set up by it. This type of action has been carefully studied by Levy in the case of epinephrine, and the resemblances between the action of tyramine and epinephrine are close enough to justify the expectation that tyramine also might start up a fibrillation of the ventricles in rare cases when great cardiac depression had been produced by arsphenamine. Auer has mentioned the unstable condition of the heart in severe cases of experimental poisoning with arsphenamine, and we have seen a few cases in which the heart finally passed into delirium cordis; and in one or two animals this seemed evidently to be, at least partly, due to the tyramine injected.

Experimentally we have found that if any remedial measures are to be employed in an attempt to save the animal, such measures must be applied early if any success is to be expected from their application.

CONCLUSIONS

1. The slow injection of therapeutic quantities of arsphenamine in very dilute, alkaline solution (monosodium salt) produces no striking results in anaesthetized dogs.

2. As the rate of injection and the concentration of the drug are increased, toxic symptoms soon begin to manifest themselves.

3. The earliest of these symptoms consist in a dilatation of the heart, perhaps mainly of the right side at first, a progressively increasing pulmonary blood-pressure, and a slow, gradual, but not severe, fall of the systemic pressure.

4. The cause of this rise in pulmonary arterial tension we believe to be due partly to the alkalinity of the solutions of arsphenamine used, and partly to the specific action of the drug itself. While we have not so far been able to prove that the formation of emboli in the pulmonary arterioles and capillaries may not be in part responsible for the increased pulmonary arterial tension, still we believe that no such action as this occurs.

5. With large toxic doses the right heart may have to contract against a pulmonary pressure increased by 100 per cent above the normal, while at the same time the left ventricle may be contracting against a systemic pressure reduced by 25 to 50 per cent below the normal. These peculiar conditions may tend to establish a state of increased irritability and instability in the heart, and in rare instances delirium cordis may result. Drugs of the epinephrine type might tend to increase the instability of the heart under these conditions.

6. The reactions of the internal organs when arsphenamine is injected are variable, and the reasons therefor are obscure. Apparently both central and peripheral influences are concerned. As a rule, we have found oncometric tracings of the spleen and intestinal loop to show a dilatation, while the kidney usually contracts, sometimes in a most vigorous fashion.

7. The toxicity of arsphenamine is not increased by the breathing of high concentrations of carbon dioxide, nor by the injection of calcium hydrate, calcium lactate, or of monosodium phosphate.

8. A number of intermediary compounds occurring during the process of manufacture of arsphenamine were studied. None of these is very poisonous and they cannot account for the variable toxicity of different samples of arsphenamine which may or may not contain traces of one or more of these.

9. The suggestion is made that in those cases in which severe, acute, toxic symptoms suddenly manifest themselves, either during or shortly after the intravenous injection of arsphenamine, tyramine is more likely to be of benefit to the patient than is any other drug with which we are acquainted.

REFERENCES

- EHRLICH AND BERTHEIM: *Chem. Beitr.*, 1912, xlv, 756.
AUER: *Arch. Int. Med.* 1911, viii, 169; *J. Exp. Med.*, 1911, xiv, 248; *ibid.*, 497.
ALWENS: *Arch. f. Exp. Path. & Phar.*, 1913, lxxii, 177.
LUTHLEN: *Ztschr. f. Exp. Path. & Ther.*, 1913, xiii, 495.
SCHLASBERG: *Nord. Med. Arkiv.*, 1913, xlv, no. 2, 1.
FLEIG: *La toxicité du salvarsan* (Maloine, Ed., 1914).
HOKE AND RIHL: *Ztschr. f. Exp. Path. & Ther.*, 1911, ix, 332.
BURZI: *Riforma Medica*, 1912, xxviii, no. 11.
JOSEPH: *J. Exp. Med.*, 1911, xiv, 83; *ibid.*, p. 179.
ROTH: *Bull. No. 113, Hyg. Lab., Wash.*
MYERS AND DuMEZ: *Public Health Reports*, June, 1918, p. 1003.
DANYSZ: *Ann. de l'Inst. Pasteur, Paris*, 1917, no. 3, p. 114.
MANN, F. C.: *Surg., Gynec. & Obst.*, Chicago, 1915, xxi, 430.
SEELIG AND JOSEPH: *J. Lab. & Clin. Med.*, 1916, i, 283.
HEWLETT, A. W.: *Arch. Int. Med.*, 1918, xxi, 411.

THE MODE OF ACTION OF CERTAIN STIMULANTS IN INCREASING AND OF CERTAIN DEPRESSANTS IN DECREASING OXIDATION

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It is known that oxidation is increased during the action of certain stimulants and decreased during the action of certain depressants. Hoppe (1), Smith (2), Reichert (3), Edsall and Means (4), Higgins and Means (5), Means, Aub, and DuBois (6), found that stimulants such as coffee, tea, and cocoa, or the active principles of these beverages, caffein and theobromin, increased oxidation in the body. Alexander and Cserna (7) showed that oxidation was increased during the excitement stage of depressants, such as narcotics, while Paul Bert as well as Arloing (8) showed that after the preliminary stimulating action had passed, the effect of the narcotics was to produce a decrease in oxidation, and that this decrease was more marked with a powerful anaesthetic, such as chloroform, than with a less powerful anaesthetic, such as ether.

The present investigation was begun in an attempt to determine how stimulants, such as caffein and theobromin produce an increase, and how depressants, such as chloroform, ether, nitrous oxide, and magnesium sulphate produce a decrease in oxidation. We had already found that whatever increased oxidation in the body produced a corresponding increase in catalase by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation produced a corresponding decrease in catalase by decreasing the output from the liver and by direct destruction of the enzyme. It was found, for example, that the ingestion of food increased the catalase of the blood, and hence of the tissues,

parallel with the increase in heat production by stimulating the digestive glands, especially the liver, to an increased output of this enzyme; that the stimulating effect of meat was due to the amino-acids, that of fat to the glycerin radical of the fat molecule, and of starchy foods to the simple sugars (9). It (9) was also found that the liver was stimulated over the splanchnic nerves during combat to an increased output of catalase parallel with the increase in heat production, and that catalase was decreased during "shock" parallel with the decrease produced in oxidation. From these and similar observations, the conclusion was drawn that it is probable that catalase is the enzyme principally responsible for oxidation in the body. Stated more specifically, the present investigation was begun in an attempt to determine if stimulants, such as caffein and theobromin, produce an increase in catalase parallel with the increase in heat production, by stimulating the digestive glands, especially the liver, to an increased output of this enzyme, and if depressants, such as chloroform, ether, nitrous oxide, and magnesium sulphate, produce a decrease in catalase parallel with the decrease produced in heat production by decreasing the output from the liver and by the direct destruction of the enzyme.

The animals used were dogs, rabbits, and cats. The catalase in 0.5 cc. of blood was determined before as well as at fixed intervals after the administration of the substances named. The determinations were made by adding 0.5 cc. of blood to diluted hydrogen peroxide, and after reducing the volume of oxygen gas liberated in ten minutes to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood.

In figure 1 are given curves showing the effect of chloroform, nitrous oxide, ether, magnesium sulphate, theobromin, and caffein on the catalase content of the blood. The chloroform and ether were administered by bubbling air through these anaesthetics in a bottle which was connected by a rubber tube to a cone adjusted to the snout of the animal; nitrous oxide anaesthesia was produced by administering a mixture of nitrous oxide and oxygen in the proportion of 1 to 5; the magnesium sulphate

anaesthesia, by the subcutaneous injection of 7.5 cc. of a 20 per cent magnesium sulphate solution per kilogram of body weight.

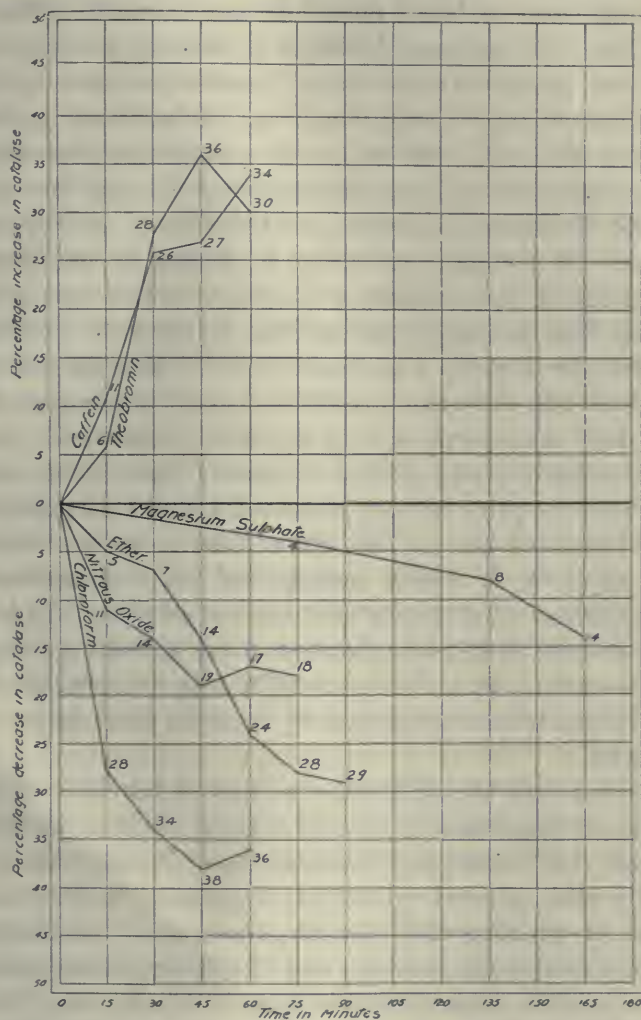


FIG. 1. CURVES SHOWING THE PERCENTAGE DECREASE PRODUCED IN THE CATALASE OF THE BLOOD BY THE NARCOTICS, AND THE PERCENTAGE INCREASE PRODUCED BY CAFFEIN AND THEOBROMIN

No attempt was made to administer these anaesthetics in equimolecular concentrations. They were administered in sufficient concentrations to produce a fair degree of anaesthesia by the end

of the first fifteen minute interval, and the amount administered during the remaining period was such as to keep the animals, rabbits and cats, in fairly deep but safe narcosis. The figures (0-50) along the ordinate indicate percentage decrease in catalase and the figures (0-180) along the abscissa, time in minutes. The catalase of 0.5 cc. of blood from the external jugular was determined before as well as at fifteen minute intervals during the administration of the anaesthetics. It may be seen that chloroform decreased the catalase of the blood 28 per cent, nitrous oxide 11 per cent, and ether 5 per cent during the first fifteen minutes of anaesthesia, and at the end of thirty minutes chloroform had decreased the catalase 34 per cent, nitrous oxide 14 per cent, and ether 7 per cent. If the decrease in catalase produced by the different anaesthetics used be compared it will be seen that chloroform, a very powerful anaesthetic, produced the most extensive and abrupt decrease; that nitrous oxide, in keeping with its quick action, produced a rapid decrease, but not such an extensive one as did chloroform or ether, while magnesium sulphate, a slowly acting and weak anaesthetic, decreased catalase very slowly and not extensively. It is known that chloroform decreases oxidation more extensively than ether or nitrous oxide. From the curves, it may be seen that it produces a more extensive decrease in catalase than either of these anaesthetics.

After reviewing the different theories of narcosis, Hewitt, in his book on *Anaesthetics* (1907), states "that it is not at all improbable that future experimental research may lead us to the conclusion that general anaesthetics produce their characteristic effect by limiting the normal processes of oxidation, upon which the intellectual, sensory, and motor centers depend for the execution of their respective functions." So far as I have been able to find, John Snow, in his classical work, *On Chloroform and Other Anaesthetics* (1858), was the first to suggest that narcotics may produce narcosis by limiting or interfering with the normal oxidative processes. Verworn (10), however, is the one who has furnished most evidence and contended most strongly that narcosis is due to decreased oxidation. If narcosis

is due to decreased oxidation, the experiments, just described suggest that this is probably due to the decrease in catalase.

The antagonistic action of caffein and the narcotics is recognized. If the depression produced by narcotics is due to a decrease in oxidation brought about by a decrease in catalase, then caffein and theobromin should produce an increase in catalase with resulting increase in oxidation. The following experiments were carried out to determine if this was true.

After making a small incision, during ether anaesthesia, in the abdominal wall of dogs 0.15 grams per kilogram of body weight of caffein in concentrated solution, as well as of theobromin were introduced by means of a hypodermic syringe into the upper part of the small intestine of the animals. The wound was then closed and the ether discontinued. Before as well as at fifteen minute intervals after introducing the material into the intestine, the catalase content of 0.5 cc. of blood from the external jugular vein was determined. The results are shown in the curves marked "theobromin" and "caffeine" in chart 1. It may be seen that theobromin increased the catalase of the blood of the jugular during the first fifteen minute interval 6 per cent, and the caffeine 11 per cent. During the second fifteen minute interval, or after thirty minutes, the theobromin had increased the catalase 28 per cent, and the caffeine 26 per cent; and after forty-five minutes, the theobromin had produced an increase of 36 per cent, and caffeine of 27 per cent. In view of these results, the antagonistic action of caffein and theobromin to the action of the narcotics would seem to be due to their opposing effect on the production of catalase.

The object of the second part of this paper was to determine the mode of action of stimulants, such as caffein and theobromin, in producing an increase, and of depressants, such as the narcotics, in producing a decrease in catalase. We had already observed that the catalase content of the blood of the liver was always 15 to 20 per cent higher than that of the blood from any other part of the body. This observation was interpreted to mean that the liver was continually replenishing the blood and hence the tissues with catalase. We had also found that after the in-

gestion of food the catalase of the blood of the liver was increased more rapidly than that from any other part of the body. This observation was taken to mean that the absorbed food materials, being carried to the liver, were stimulating this organ to an increased output of catalase. It was also found that when the liver was cut out of the circulation by means of an Eck fistula and by tying off the hepatic arteries, the introduction of ethyl alcohol into the alimentary tract produced a very small or no increase, whereas normally this substance produces a large increase in catalase. From these observations, the conclusion was drawn that the liver is the principal organ in which catalase is formed and given off to the blood. Stated more specifically the second part of this paper concerns itself with determining if the increase in catalase after the ingestion of caffein and theobromin is due to the stimulation of the liver to an increased production of this enzyme and if the decrease in catalase during anaesthesia is due to the decreased output from the liver and to the direct destruction of this enzyme by the narcotics.

The effect of chloroform and ether anaesthesia on the catalase content of the blood of the liver and of the jugular vein is shown in figure 2 under "chloroform" and "ether." The blood from the liver was obtained from a superficial incision made in this organ. The chloroform and ether were administered to the cats as described in the first part of the paper. The continuous line curves show amounts of catalase in the blood of the liver and the discontinuous line curves the amounts in the blood of the jugular. It will be seen under "chloroform" in figure 2, that 0.5 cc. of blood from the liver at the beginning of the experiment with this anaesthetic liberated 620 cc. of oxygen from hydrogen peroxide in ten minutes, while a similar amount of blood from the jugular liberated 540 cc. After fifteen minutes of chloroform anaesthesia, the catalase content of the blood of the liver was identical with that of the jugular as is indicated by the fact that 0.5 cc. of blood liberated the same amount of oxygen from hydrogen peroxide, namely 490 cc. The fact that at the beginning of the experiment the blood of the liver was richer in catalase than the blood of the jugular is interpreted to mean that

the liver was putting out catalase into the blood, and the fact that after fifteen minutes of chloroform anaesthesia the catalase of the blood of the liver and of the jugular was identical and remained practical identical during the subsequent periods of anaesthesia, is taken to mean that the chloroform had decreased the output of catalase from the liver to zero. By examining the curve under "ether" it will be seen that while this anaesthetic decreased the catalase in the blood of the liver and of the jugular vein, as is indicated by the decrease in the amount of oxygen liberated from hydrogen peroxide, the catalase of the blood of the liver remained, throughout the period of administration of the anaesthetic higher than that in the blood of the jugular. This observation is interpreted to mean that the ether was not disturbing the liver function in respect to catalase production as much as did the chloroform.

That the decrease in catalase during anaesthesia is due to the direct destruction of this enzyme by anaesthetics as well as to the decreased output from the liver is shown in figure 2 under "in vitro." These curves were constructed from data obtained from defibrinated blood of a cat. Ten cubic centimeters of this blood were introduced into a vessel, and exposed to ether vapor for 60, 120, and 180 minutes, respectively, under a pressure of 40 mm. mercury at 40°C. Under identical conditions and simultaneously, a similar sample of blood was exposed to chloroform vapor. Before exposing the blood to the vapor of the anaesthetic, a determination of the catalase content of 0.5 cc. was made and it will be seen in the chart that this amount of blood liberated 600 cc. of oxygen from hydrogen peroxide in ten minutes. After sixty minutes of exposure to chloroform vapor it may be seen that 0.5 cc. of the blood liberated 380 cc. oxygen, while the blood that had been exposed to the ether vapor liberated 460 cc. By comparing these figures it may be seen that the chloroform vapor was more destructive to catalase than the ether vapor. From the preceding experiments it is evident that the decrease produced in the catalase of the blood by the narcotics is due to the decreased output of catalase from the liver and to the direct destruction of the enzyme by the narcotic.

The curves in figure 2 under "cafein" and "theobromin" show the effect of the introduction of these substances into the intestine of dogs on the catalase content of the blood of the liver and of the jugular vein. The continuous line curves represent amounts of catalase in the blood of the liver while the discontinuous line curves, the amounts in the jugular vein. The method of introduction, and amounts of caffein and theo-

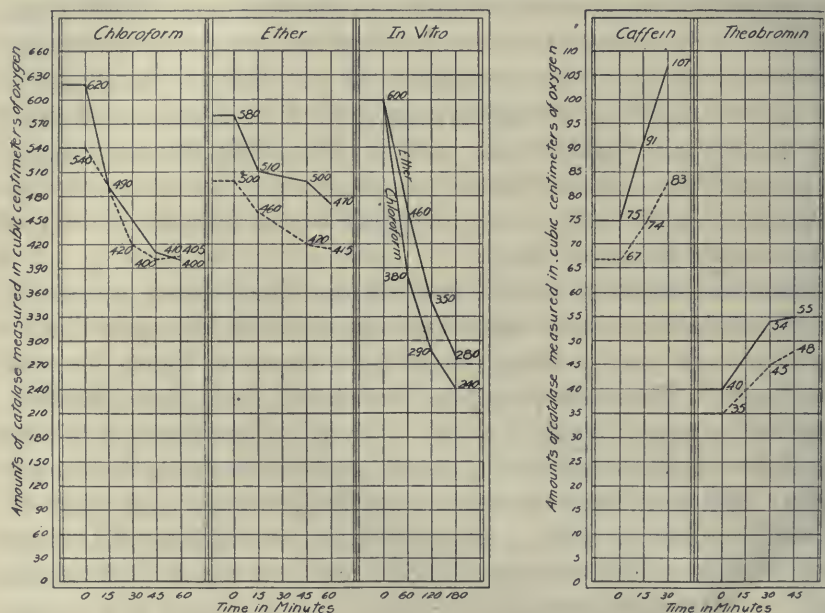


FIG. 2. CURVES SHOWING THE DECREASE PRODUCED IN THE CATALASE OF THE BLOOD OF THE LIVER AND OF THE JUGULAR VEIN BY CHLOROFORM AND ETHER AND THE INCREASE PRODUCED BY CAFFEIN AND THEOBROMIN

The continuous line curves show amount of catalase in the blood of the liver; the discontinuous line curves, the amount in the blood of the jugular vein.

bromin used were the same as those given in the first part of the paper. Under "cafein," it may be seen that previous to the introduction of 0.15 grams per kilogram of body weight of this substance, 0.5 cc. of blood from the liver liberated 75 cc. of oxygen from hydrogen peroxide, while a similar amount of blood from the jugular liberated 67 cc. Fifteen minutes after the introduction of the caffein 0.5 cc. of the blood of the liver

liberated 91 cc. of oxygen and the jugular blood 74 cc. By comparing these figures, it may be seen that the catalase in the blood of the liver was increased much more rapidly than was the blood of the jugular, which is taken to mean that the caffein was stimulating the liver to an increased output of catalase. A similar comparison of the curves under "theobromin" will show that this substance also increased the catalase of the blood by stimulating the liver to an increased output of this enzyme.

SUMMARY

1. Stimulants, such as caffein, and theobromin, produce an increase in catalase with resulting increase in oxidation by stimulating the liver to an increased output of this enzyme, while depressants, such as the narcotics produce a decrease in catalase with resulting decrease in oxidation by decreasing the output from the liver and by the direct destruction of the enzyme. A powerful anaesthetic, such as chloroform, is more effective in decreasing the output of catalase from the liver and in destroying this enzyme in vitro than a less powerful anaesthetic such as ether. A quickly acting anaesthetic such as nitrous oxide decreases catalase more quickly, but less extensively than does a slower acting but more powerful anaesthetic such as ether.

2. Evidence is presented in this paper to show that depressants such as the narcotics decrease oxidation which may be the cause of the depression by producing a decrease in catalase, while stimulants such as caffein and theobromin produce an increase in oxidation which may be the cause of the stimulation by producing an increase in catalase.

3. The fact that the action of caffein and theobromin is to produce an increase in catalase with resulting increase in oxidation while the action of the narcotics is to produce a decrease in catalase with resulting decrease in oxidation is offered as an explanation for the antagonistic action of these substances.

REFERENCES

- (1) HOPPE: *Deutsch. Klin.*, 1857, ix, 181.
- (2) SMITH: *Philosophical Transactions*, London, 1859, cxlix, 681.

- (3) REICHERT: University Med. Mag., Philadelphia, 1890, ii, 173, 225, 345.
- (4) EDSALL AND MEANS: The Archives of Int. Med., 1914, xiv, 897.
- (5) HIGGINS AND MEANS: Jour. Pharmacol. and Exper. Therap., 1915, vii, 1.
- (6) MEANS, AUB, AND DuBOIS: Archives Int. Med., 1917, xix, 832.
- (7) ALEXANDER AND CSERNA: Biochem. Zeitschr., 1913, liii, 101.
- (8) BERT AND ARLOING: Dastre, Les Anaesthesiques, Paris, 1890.
- (9) BURGE AND NEILL: Jour. of Physiology, 1918, xlvii, 1, 13.
BURGE: Jour. of Physiology, 1917, xlv, 3, 290.
BURGE AND NEILL: Jour. of Physiology, 1918, xlv, 3, 286.
- (10) VERWORN: Narkose, Jena, 1912; Narcosis, Harvey Lect., 1912, 152.

ADENINE MONONUCLEOTIDE

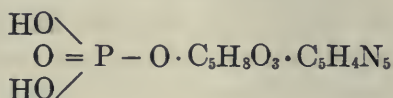
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We have prepared from yeast nucleic acid a crystalline substance whose elementary analyses lead sharply to the formula $C_{10}H_{14}N_5PO_7 \cdot H_2O$. It is quite soluble in hot water and very difficultly soluble in cold water so that its repeated crystallization does not involve any serious loss of material.

The compound responds to the pentose color reactions, forms a brucine salt containing two equivalents of brucine and by hydrolysis with dilute sulphuric acid yields adenine but no guanine. Its entire phosphoric acid is "easily split" so that it contains neither a cytosine nor a uracil group. The compound is evidently adenine mononucleotide



and its chemical conduct is in agreement throughout with that which has been predicted for it.

The existence of a group in nucleic acid corresponding to adenine mononucleotide has been conceded on indirect evidence but the substance has never been actually prepared from yeast nucleic acid. The physical properties of the mononucleotide as well as those of its derivatives are therefore known now for the first time, and it is interesting to note that we prepared the substance from its brucine salt which had been recrystallized ten times from hot 35 per cent alcohol. A detailed publication will follow shortly.

ON THE PHARMACOLOGY OF THE URETER

VI. ACTION OF SOME OPTIC ISOMERS¹

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A difference in physiological activity on the part of different optical isomers of substances which are in other respects chemically absolutely identical has been noted by a number of authors. Thus Cushny (1) found a difference in pharmacological action between the three varieties, laevo- dextro- and inactive, of hyoscyamin. Cushny and Peebles (2) further studied the different effects of the optic isomers of hyoscin or scopolamin. Flaecher and others (3) noted a difference in physiological activity between the optic isomers of epinephrin or adrenalin. Grove (4) reported a difference in toxicity between the ordinary or dextro form and the other optical varieties of camphor. In a preceding paper dealing with the action of atropin on the isolated ureter (5) it was found that small doses of atropin *stimulated* the contractions of that organ while larger doses of the same drug produced an *inhibition* of the ureteral contractions. Inasmuch as atropin is the inactive or racemic form of hyoscyamin, it was deemed very desirable to ascertain the action of the two optically active varieties of hyoscyamin on the ureter as compared with that of atropin. As far as the author has been able to learn, no systematic study of the effects of various optic isomers on isolated smooth muscle tissue has been made by any of the authors above cited. Inasmuch as the isolated ureteral rings afford a very convenient and very accurate, almost quantitative, method of recording the rhythmic contractions of plain muscle, the author undertook to study the action of a number of optical isomers on that organ.

¹ The observations here published were first reported by the author before the Joint Session of the American Pharmacological, American Physiological, American Biochemical, and American Pathological Societies, held in New York, December 28, 1916.

SUBSTANCES STUDIED

The method employed has been already described in the preceding papers on the pharmacology of the ureter. Four groups of optic isomers were studied by the author, namely, the three varieties of hyoscyamin, the three varieties of hyoscin or scopolamin, the laevo and dextro forms of epinephrin and the three optical varieties of camphor. The laevo forms of the various alkaloids could, of course, be easily obtained in the laboratory. The synthetic or inactive form of epinephrin happened also very fortunately to be in the possession of the author.

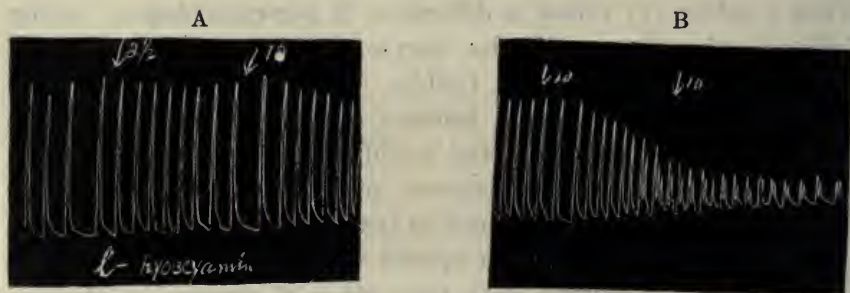


FIG. 1. PIG'S URETER

A. Laevo-hyoscyamin ($\alpha [D] = -20^\circ$) in doses of 2.5 mgm. to 22.5 mgm. in 25 cc. Locke's solution produces stimulation.

B. Ten minutes later, an additional dose of laevo-hyoscyamin produces secondary depression.

For the dextro forms of hyoscyamin and hyoscin the author is indebted to the kindness of Prof. A. Cushny and grateful acknowledgment for the same is hereby made. The laevo and synthetic or inactive forms of camphor were obtained through the courtesy of Professor Kremers of Wisconsin, to whom the author is also gratefully indebted.

ACTION OF HYOSCYAMINS

Several specimens of laevo-gyrous hyoscyamin hydrochloride and hydrobromide were examined. These varied in their

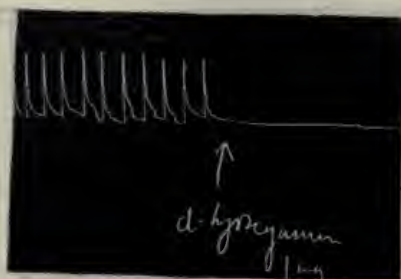


FIG. 2. PIG'S URETER

Dextro-hyoscyamin-dextro-campho-sulphate (by courtesy of Professor Cushny) inhibits even after a small dose, 1 mgm. in 25 cc. Locke's solution.

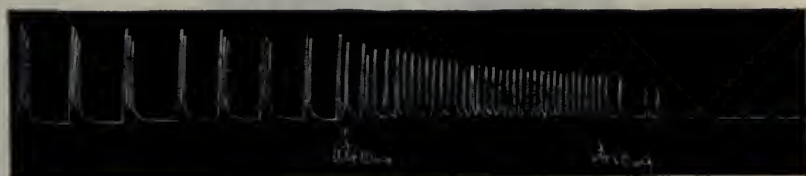


FIG. 3. RING OF PIG'S URETER

Twenty-four hours after excision. Primary stimulation of frequency of contractions and subsequent inhibition after atropin.

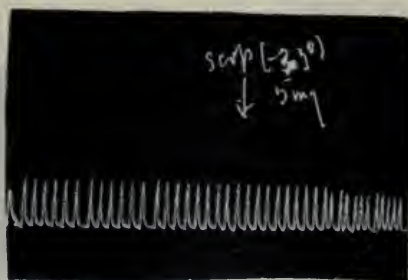


FIG. 4. PIG'S URETER

Laevo-hyoscin ($\alpha_{[D]} = -23^\circ$) 5 mgm. in 25 cc. of Locke's solution, produces stimulation.

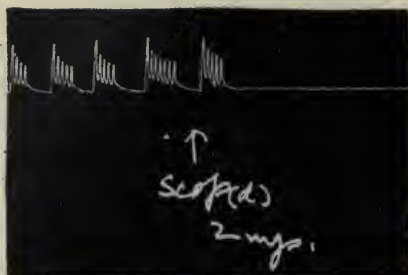


FIG. 5. PIG'S URETER

Dextro-hyoscin, 2 mgm. in 25 cc. of Locke's solution produces inhibition.

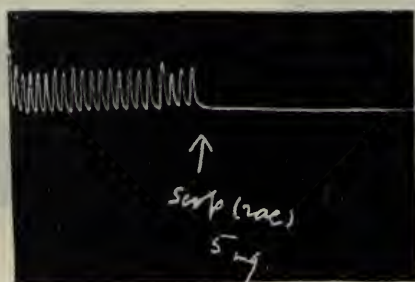


FIG. 6. PIG'S URETER

Racemic or inactive scopolamin produces inhibition.



FIG. 7. PIG'S URETER

Euscopol, an inactive scopolamin produces gradual inhibition.

degree of rotation. All the specimens of laevo-hyoscyamin were found to stimulate the contractions and to slightly increase the tonus of the ureter. It was only after very large doses of that drug that the contractions of the ureter were depressed (fig. 1). The dextro-gyrous form of hyoscyamin, on the other hand, was found not to stimulate the contractions of the ureter but tended to inhibit them from the start (fig. 2). Experiments with atropin or the inactive form of hyoscyamin gave a result standing midway between those of the two active varieties. The primary stimulating effect of small doses of atropin may therefore be regarded as due to the preponderating effect of the laevo-gyrous component on the ureter while the secondary inhibition of the contractions by large doses of atropin may be

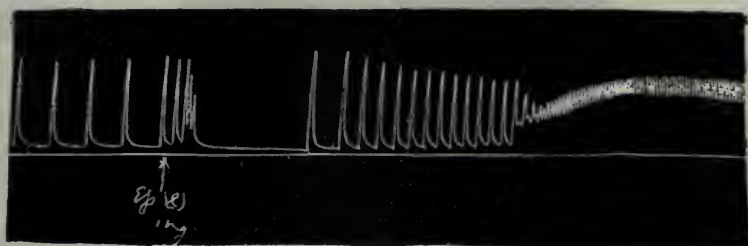


FIG. 8. PIG'S URETER

Action of laevo epinephrin ($\alpha_{[D]} = -51^\circ$) 1 mgm. in 25 cc. Locke's solution.

ascribed to the effect of the dextro-gyrous component (fig. 3). At any rate, the difference between the stimulating action of the laevo variety and the inhibitory action of the dextro variety is very striking, as seen in the figures (figs. 1 and 2).

THE ACTION OF HYOSCINS OR SCOPOLAMINS

Just as in the case of hyoscyamin, so in case of hyoscin or scopolamin, the laevo variety was found to stimulate while the dextro variety was found to inhibit the contractions of the ureter. The effect of the inactive or racemic form of scopolamin was also an inhibitory one; thus showing that the dextro component exerted the upper hand (figs. 4, 5, 6, 7).

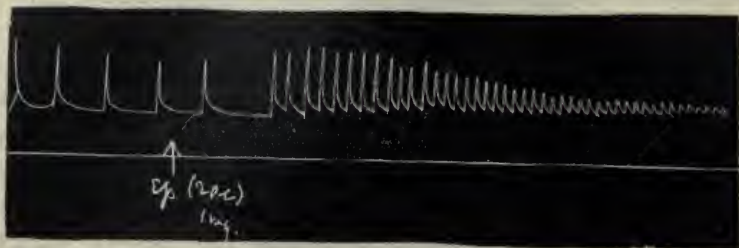


FIG. 9. PIG'S URETER

Action of synthetic or inactive epinephrin, 1 mgm. in 25 cc. Locke's solution.

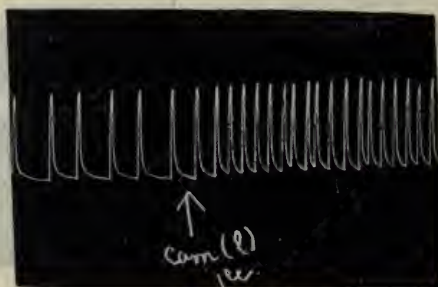


FIG. 10. PIG'S URETER

Action of laevo-camphor 1:10000 solution.



FIG. 11. PIG'S URETER

Action of dextro camphor 1:10000 solution.

ACTION OF EPINEPHRIN

The effect of the laevo and synthetic forms of epinephrin upon the ureter corresponded to the action of the two varieties on other physiological functions described by various authors and was not a qualitative but a quantitative one. The laevo-rotatory variety was found to be much more active and stimulated the

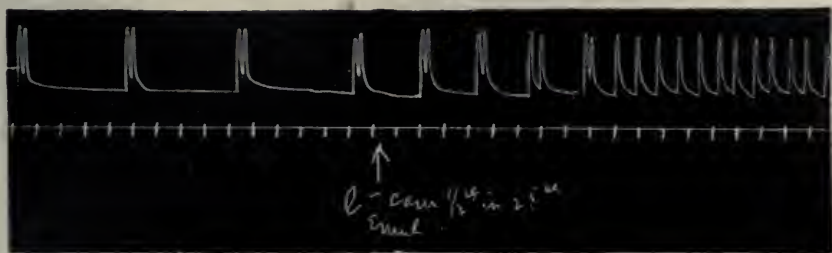


FIG. 12. PIG'S URETER

Action of 1 per cent emulsion of laevo-camphor; 0.5 cc. in 25 cc. Locke's solution. Note marked stimulation.

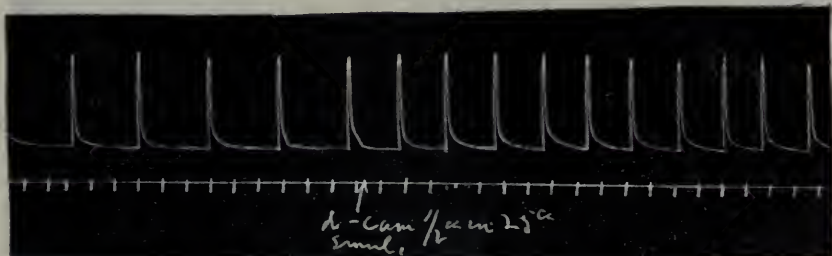


FIG. 13. PIG'S URETER

Action of 1 per cent emulsion of dextro-camphor; 0.5 cc. in 25 cc. Locke's solution. Note why slight stimulation.

ureteral contractions and raised the ureteral tonus much more than the synthetic or racemic variety. So marked was the difference in the degree of stimulation that the author could easily determine which one of two unknown solutions of epi-

nephrin contained the laevo variety and which contained the synthetic variety (figs. 8 and 9). The author could not secure a specimen of dextro epinephrin.

Action of camphors

The effect of camphor on the contractions of the ureter was studied in two ways. Camphor was found to be sufficiently soluble in normal saline solutions to produce an appreciable effect on the isolated ureteral rings. Accordingly solutions of the strength of 1 to 20,000 of the three varieties were studied. It was found that laevo camphor produced a marked stimulation of the ureteral contractions, while the dextro variety in the same

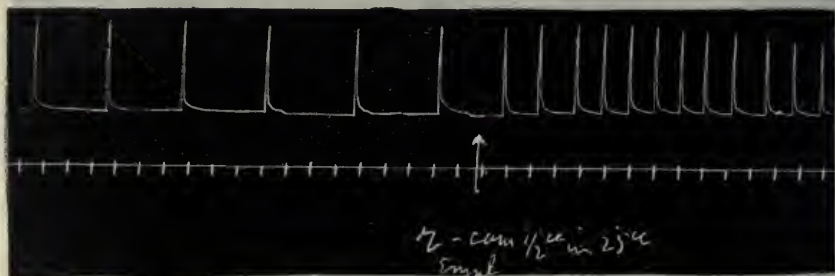


FIG. 14. PIG'S URETER

Action of 1 per cent emulsion of racemic or optically inactive (synthetic) camphor; 0.5 cc. in 25 cc. Locke's solution. Note effect half way between the laevo and dextro varieties.

doses produced little or no effect. The effect of the racemic variety was found to be the arithmetical mean of the two active components (figs. 10 and 11).

The action of camphor was furthermore studied by the use of emulsions or suspensions in oil and acacia. The results were exactly the same as those given by the aqueous solutions (figs. 12, 13, and 14).

DISCUSSION

From the above described experiments it is seen that the different optical varieties of the four groups of drugs studied affect

the contractions of the ureter differently To use the words of Cushny, "Some organs have the property of differentiating between optical isomers." In the case of the ureter, we have a very striking method of demonstrating such a difference in connection with the action of optic isomers on smooth muscle. It is further interesting to note that in case of all the groups of isomers studied the laevo-gyrous variety was the more stimulating one. The explanation of this phenomenon, of course, is not at hand.

SUMMARY

1. The action of four groups of optic isomers on the isolated ureter were studied.

2. It was found that in case of the different optic varieties of hyoscyamin, scopolamin, epinephrin and camphor the pharmacological action was different.

3. It was further found that the laevo-gyrous variety in each case was the more stimulating than the dextro-gyrous one.

REFERENCES

- (1) CUSHNY: Jour. of Physiol., xxx, 176, 1904.
- (2) CUSHNY AND PEEBLES: Jour. of Physiol., xxxii, 501, 1906.
- (3) FLÄCHER: Ztschr. f. physiol. chem., lviii, 189, 1909.
- (4) GROVE: Jour. of Pharmacol. and Exp. Therap. i, 445, 1909.
- (5) MACHT: Jour. of Pharmacol. and Exp. Therap., viii, 261, 1916.

ON DICHLORETHYLSULPHIDE (MUSTARD GAS)

I. THE SYSTEMIC EFFECTS AND MECHANISM OF ACTION

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I. INTRODUCTION

The use of dichlorethylsulphide or the now well known mustard gas in warfare, has given rise to considerable interest regarding this substance (1). The present communication deals with three important points: (1) Proof of the absorption of the

¹ Vernon Lynch was associated in this work very actively, and his death in July, 1918 was a great loss to the work and to science.

substance into the blood stream through the lungs and skin, (2), a description of the systemic effects as apart from the local action on the respiratory tract, eyes, and skin, and (3), evidence for a theory of the mechanism of the action of the substance.

Dichlorethylsulphide was first prepared by Victor Meyer (2). It is a colorless, oily liquid, boiling at about 217°C ., only very slightly soluble in water, but very soluble in all organic solvents. It has a very characteristic odor, suggestive of mustard or garlic. Its discoverer recognized its poisonous effects on the respiratory tract, skin and eyes.

When an animal is exposed to the vapors of dichlorethylsulphide (3) in high concentration it subsequently shows a complex of symptoms which may be divided into two classes:

1. The local effects on the eyes, skin, and respiratory tract. These are well recognized and consist mainly of conjunctivitis and superficial necrosis of the cornea; hyperemia, edema, and later necrosis of the skin, leading to a skin lesion of great chronicity; and congestion, and necrosis of the epithelial lining of the trachea and bronchi.

2. The systemic effects due to the absorption of the substance into the blood stream and its distribution to the various tissues of the body. These effects are not generally recognized, and will be discussed in this article.

The most striking observation about the symptoms of dichlorethylsulphide poisoning is the latent period which elapses after exposure before any serious objective or subjective effects are noted. The development of the effects are then quite slow, unless very high superlethal doses have been inhaled.

The symptoms which are observed upon dogs when subjected to the vapors of dichlorethylsulphide which might suggest absorption into the blood stream and a systemic effect, are as follows: Vomiting and diarrhea, hyperexcitability and convulsions, and effects upon the heart. Moreover, the condition of the lungs and trachea found at autopsy of some animals dying from the inhalation of the gas, is not sufficient to account for death.

II. ABSORPTION THROUGH THE LUNGS AND SYSTEMIC EFFECTS

1. *Injections of dichlorethylsulphide.* In order to become familiar with the effects of the absorption of dichlorethylsulphide into the system, dogs were injected with the substance. The simplest method of introducing the substance is by subcutaneous or intramuscular injection of olive oil solutions. The effects observed on an unanaesthetized animal from the injection of a lethal dose in olive oil, are, after a latent period: salivation, hyperexcitability and convulsions, diarrhea, slow and irregular heart which becomes rapid before death, muscular weakness, and finally coma and death.

The following protocols illustrate the effects of subcutaneous and intramuscular injections in olive oil solution:

Experiment 1. Dog V7, male; weight, 12.5 kilos.

March 12.

10.15 Pulse 136, respiration 18.

10.28 Subcutaneous injection of 500 mgm. dichlorethylsulphide in 5 grams olive oil.

10.45 Behavior normal. Licks point of injection.

11.00 Somewhat restless.

12.00 Salivation, vomiting, diarrhea.

1.15 Pulse 66, respiration 32.

1.30 Muscular spasms, especially in hind legs. Convulsions. Animal staggers. Finally unable to walk.

1.40 Intraperitoneal injection of 3 grams chloretone in olive oil.

2.10 Pulse 66 (very strong), respiration 56. Still excitable.

2.45 Pulse 64, respiration 36.

4.15 Pulse 126, respiration 26. Given another injection of 500 mgm. sulphide.

5.00 Animal conscious, but unable to stand.

March 13. Found dead.

Autopsy. Lungs and trachea appear normal; stomach contains bile and bloody fluid; intestines congested with few areas of hemorrhage and contain bloody fluid.

Experiment 2. Dog V10, female; weight, 10 kilos.

9.50 Pulse 88, respiration 18.

- 9.55 Subcutaneous injection of 1000 mgm. of dichlorethylsulphide in 10 cc. of olive oil, 5 cc. on each side.
- 10.00 Pulse 90, respiration 20. Animal quiet.
- 10.15 Restless.
- 10.30 Pulse 118, respiration 44.
- 10.50 Pulse 112, respiration very rapid, slight salivation.
- 11.15 Salivation, rapid respiration, diarrhea.
- 12.00 Pulse 44, respiration 102. Animal has previously had very good control of muscles. Movements are now stiff, convulsive, and uncontrolled. Tries to struggle to feet, but topples over and paws ground convulsively.
- 1.00 Pulse 36 (very strong, but irregular), respiration 112.
- 1.45 Pulse 36, respiration 64. Very weak, unable to rise. Has vomited.
- 2.40 Pulse 54, respiration 82. Vomiting. Now perfectly quiet.
- 3.00 Pulse 72, respiration 66.
- 3.20 Pulse 108 (very weak), respiration 54.
- 3.30 Dies.

Autopsy. Trachea and lungs appear slightly congested; stomach filled with blood fluid; intestines congested and hemorrhagic; other organs appear normal.

Experiment 3. Dog DM203; weight, 17 kilos.

September 23.

- 10.30 Pulse 88, respiration 18.
- 10.35 Intramuscular injection of 240 mgm. (14 mgm. per kilo) of dichlorethylsulphide in 12 cc. of olive oil.
- 11.00 Behavior normal. Pulse 114, respiration 30.
- 11.30 No toxic symptoms as yet. Pulse 100, respiration 20.
- 12.00 Pulse 102, respiration panting; is becoming hyperexcitable; appears irritable and trembles slightly, but walks without ataxia.
- 12.45 Pulse 120, respiration 28. Walks uncertainly; salivated; pupils normal. Injected leg affected, seems sore, and dog refuses to use it in standing or walking.
- 1.10 Pulse 120, respiration 30. Injected leg no longer seems sore, but is wholly useless.
- 1.45 Pulse 130, respiration 36. Though seemingly conscious, movements are decidedly convulsive. No diarrhea, great salivation or hyperexcitability. Pupils are now greatly dilated.

2.00 Pulse 114, respiration 29. Dog exhibits twitching and slow convulsive movements. Knee reflex in injected leg about normal, the leg is inactive during convulsive movements. Very irritable and snappy at times; then again is very affectionate. After drinking heavily, pulse runs up to 150, but is very irregular.

2.45 Pulse 144, respiration 60. Shows slight salivation.

4.20 Pulse 174, respiration 66. Still shows slight convulsive tendencies.

September 24.

9.00 Pulse 192, respiration 48. Dog is conscious, but trembles greatly. No salivation or evidence of diarrhea. Pulse varies greatly during day.

September 25.

1.00 No marked change in condition. Pulse running 150 to 180 throughout day, respiration 24 to 35.

September 26.

10.20 Pulse 156, respiration 32.

4.00 Dog found dead seventy-seven hours after injection.

Autopsy. Conjunctivitis; slight cutaneous edema at site of injection. Tracheal blood vessels congested; *lungs* apparently normal, with slight P. M. changes. No hemorrhages in adrenal cortex. *Liver, kidneys* and *spleen* normal; mucous membranes of gut, hemorrhagic and bloody, chiefly in upper tract; *stomach* normal.

Experiment 5. Dog DM202; weight, 11 kilos.

September 23

10.30 Pulse 102, respiration 66.

10.40 Intramuscular injection of 220 mgm. (20 mgm. per kilo) of dichlorethylsulphide in 5.5 cc. of olive oil.

11.00 Dog appears normal, except for panting. Pulse 138.

11.30 Pulse 100, respiration still panting.

12.00 Pulse 78, respiration 60. Marked salivation; appears spasmodically ataxic, with severe tremors; distinctly hyperexcitable.

12.45 Pulse 78, respiration very irregular. Salivation, but no diarrhea or vomiting. Exhibits severe convulsions. Pupils dilated. Injected leg stiff and does not enter into convulsive movements, which are of a clonic nature; between convulsive spasms, dog attempts to rise, but both rear legs are inactive.

- 1.10 Pulse 75, respiration 70. Passes small quantity of semi-liquid stool without blood stain.
- 1.45 Pulse 68, respiration 36. No continued diarrhea.
- 2.00 Pulse 66, respiration 30. Heart sounds hammer-like, but very irregular in periodicity and intensity.
- 2.15 Pulse 90, respiration 28. Dog attempts to drink with great effort, but cannot rise or put nose in water. Continues to lap air. Portrays marked muscular weakness and incoordination.
- 2.30 Pulse 68, respiration 39. Eyelids twitching. Reflexes abnormally active. Heart irregular, missing every third beat.
- 2.45 Pulse 150, respiration 40.
- 3.00 Pulse 150, respiration 48. Heart sounds becoming very faint, with increased rate.
- 4.00 Pulse 162, respiration 38. Great muscular weakness. Almost unconscious. Seems to be passing into coma.
- 4.20 Pulse 192, respiration 48. Dog lies in coma.
- September 24.

12.00 Found dead after thirteen hours.

Autopsy. Trachea slightly congested. Lungs show small circumscribed points of hemorrhage throughout; considerable P. M. congestion; adrenals show slight cortical inflammation. Kidneys normal, except for slight P. M. congestion. External gut has too much P. M. change to describe extent of external hemorrhage. Stomach is slightly congested. Very marked hemorrhage in the lumen of jejunum and duodenum; entire gut blood stained throughout. Injected leg showed great subcutaneous edema, but no muscular changes beyond a slightly brighter color than in normal leg.

The intravenous injection is much more instructive but somewhat more difficult. The slight solubility of the substance in water (about 0.07 per cent at 10°C.), and the rapidity with which an aqueous solution hydrolyzes, are the main difficulties. This cannot be overcome by the injection of an alcohol or acetone solution, for as soon as these solutions come in contact with water the sulphide is precipitated out as fairly large oil droplets. These difficulties have been overcome by the injection of large amounts of a *freshly prepared, cold, saturated aqueous solution.*

The method of preparing the aqueous solution for intravenous injection is as follows: Five hundred cubic centimeters of 0.8 per cent saline is cooled to 8 to 10°C. This is placed in a flask, about 1 cc. of pure dichlorethylsulphide is added, the flask tightly stoppered and shaken for about one minute. The contents of the flask are transferred to a separatory funnel, and the oil allowed to settle. An oil film is present on the surface as well as globules at the bottom. About 400 cc. of solution is removed from between the oil and film, being careful to obtain a solution free from oil films or droplets. This is placed in a bath at 8 to 10°C. and used as soon as possible. The solution prepared in this way contains about 0.7 mgm. per cubic centimeter. The hydrolysis of the aqueous solution is a monomolecular reaction. At 10°C. only about 15 per cent of the disulphide is hydrolyzed in ten minutes, while at 37.5°C. over 97 per cent is decomposed in the same time (4).

The symptoms elicited from an intravenous injection are similar to those observed from the injection of olive oil solutions. While the solution is being injected, and for some time afterwards, the animal shows no effects, whatever. A record of the blood pressure, pulse, and respiration fails to show any appreciable effect at this time.

Within ten to twenty minutes after injection, however, an increased salivation is noticed. This soon develops into a very free flow of rather mucinous saliva. The next symptom observed is usually a diarrhea which may be accompanied by vomiting. This diarrhea is present until the death of the animal, and a few hours after the injection the stools frequently contain blood. After injection the respiration becomes rapid and if anaesthesia has not been used, the animal shows a distinct hyperexcitability. At this stage, he may be frightened by a slight movement of the hand or unexpected touch, and the eye reflex may be obtained by touching almost any part of the face. The gait soon becomes unsteady, movements of the muscles are spasmodic but uncontrolled, and apparently accompanied by tetanic contractions of the antagonist. The knee jerk may be elicited by a touch of the finger. The animal soon becomes

unable to walk or even to stand, and the violent spasmodic movements increase to the stage of convulsions, with extension of the hind legs and arching of the neck and back. The pulse, which may have been somewhat slowed, soon becomes irregular. Palpation suggests that the heart is dropping a beat occasionally. The dropped beat becomes more and more frequent until finally the heart is beating at one-half its former rate. If the chest is opened it may be clearly seen that the ventricles are beating one to every two beats of the auricles. Later the rhythm may even become 1 to 3. Stimulation of the vagi shows some apparent hyperexcitability. The bloodpressure very slowly falls, and a few hours before death, the heart resumes its normal rate. At this stage, or before, it is found that the heart can not be slowed or in any way affected by strong stimulation of the vagi, although the respiration is easily inhibited. Section of the vagi has no effect upon the heart rate except a slight slowing. Apparently the vagus endings are paralyzed. The heart becomes feeble, there is a great dilatation in the splanchnic area, a high venous pressure, and the arterial blood pressure is falling slowly. The convulsions have ceased, and the animal lies in a coma. Death comes quietly in less than twenty-four hours after the injection. It is probably due to the weakening of the heart and the great dilatation of the vessels in the splanchnic area. Autopsy reveals a more or less intense congestion of the intestinal mucosa which may extend from the pylorus to the anus, and is frequently accompanied by hemorrhage into the lumen of the intestine. The condition suggests the excretion of the dichlorethylsulphide into the intestine. These effects upon the heart, the alimentary tract, and the central nervous system are quite characteristic and unmistakable.

The following protocols are representative of the course of an intravenous injection. In all cases the cold, saturated aqueous solution, prepared as described above, was used. The solution was usually injected within ten to fifteen minutes after its preparation. When injected such a solution contained about 0.5 to 0.6 mgm. of undecomposed dichlorethylsulphide to each cubic centimeter.

Experiment 6. Dog V9, female; weight, 8.2 kilos.

11.40 Pulse 88, respiration 16.

11.50–12.00 Injection into jugular vein of 120 cc. fresh aqueous solution (14 cc. per kilo).

12.05 Shivering. Pulse 160, respiration 66.

12.20 Slight salivation, has vomited, Pulse 70, respiration 74.

12.45 Diarrhea, no blood. Pulse 52 and irregular, respiration 108.

1.20 Highly excitable, convulsions, readily thrown into spasms—comparatively quiet between.

1.45 Pulse 80, respiration 110.

2.15 Pulse 86, respiration 120.

2.45 Pulse 84, respiration 120.

3.45 Pulse 84, respiration 110. Struggling, profuse salivation, has had diarrhea for some time.

3.50 Killed with ether.

Autopsy. *Trachea* appears normal. *Lungs* very small area of congestion in one lobe, a few emphysematous patches. *Esophagus* and *stomach* appear normal. *Duodenum*—congested areas beginning at pylorus. Small and large intestine show distinct congestion.

Experiment 7. Dog V17, female; weight, 5.5 kilos.

March 29.

3.10 Pulse 120, respiration 12.

3.12–3.20 Intravenous injection of 120 cc. of cold, aqueous solution (22 cc. per kilo).

3.25 Pulse 150, respiration 42.

3.35 Pulse 120, respiration 198. Trembling, good control of movements.

3.45 Vomiting, diarrhea.

4.05 Gait very unsteady.

4.30 Vomiting, fluid stools.

4.50 Struggling, convulsions begin, salivation.

5.00 Still in convulsions.

March 30.

9.00 Found dead.

Autopsy. *Trachea* slightly congested; *lungs* appear normal; *heart* large endocardial hemorrhages; *stomach* contains bloody fluid; *duodenum* very deep red, bloody contents; small and large intestines deeply congested.

Experiment 8. Dog V36, male; weight, 9.5 kilos.

April 22.

10.45 Pulse 90, respiration 11.

11.02–11.05 Injection of 95 cc. fresh, aqueous solution into jugular vein.

11.16 Slight salivation, vomiting.

11.25 More vomiting.

11.40 Hyperexcitability.

12.45 Convulsions.

1.00 Pulse 120, respiration 30, convulsions.

In the following experiments the dose given was too small to produce convulsions, but the other symptoms are observed:

Experiment 9. Dog V20, male; weight 11 kilos.

April 2.

12.18 Pulse 120, respiration 24.

12.18–12.23 Injection of 66 cc. cold, fresh aqueous solution in jugular vein.

12.25 Pulse 116, respiration 21.

12.30 Perfectly normal.

1.05 Has vomited.

2.00 Pulse 120, respiration 20, normal movements.

3.00 Pulse 132, respiration 24, salivation, lying quietly, but leg muscle twitched a good deal.

April 3. Apparently normal, killed for autopsy.

Autopsy. Lungs appear normal; *duodenum*, congested and slightly hemorrhagic; *small intestine* contains bloody contents.

Experiment 10. Dog V34, male; weight, 7.2 kilos.

9.45 Pulse 96, respiration 12.

10.05 Intravenous injection of 36 cc. cold, fresh aqueous solution (5 cc. per kilo).

10.08 Pulse 108, respiration 14.

10.55 Has vomited.

11.05 Slight unsteadiness of gait.

2.25 Pulse 132, respiration 32. Gait normal.

Experiment 11. Dog V19, female; weight, 17.3 kilos.

April 1

12.55 Pulse 138, respiration 18.

1.00-1.05 Injection intravenously 34 cc. of cold, fresh aqueous solution.

3.00 Pulse 78, respiration 12. Trembling slightly.

5.00 Pulse 84, respiration 12, quiet.

April 2

9.00 Depressed, very quiet, refuses food. Killed for autopsy.

Autopsy. Lungs appear normal; *duodenum* congested, bloody mucous abundant; *small intestine* congested; *large intestine*, normal.

The following protocol is illustrative of the symptoms of an animal injected very slowly. The symptoms are the same as when injection is made rapidly, but appear to be somewhat more delayed and milder.

Experiment 12. Dog V16, male; weight, 4.5 kilos.

1.30 Pulse 150, respiration 18.

1.55-2.55 Intravenous injection of 100 cc. of cold, fresh, aqueous solution (22 cc. per kilo).

2.54 Pulse 126, respiration 48, slight salivation.

3.00 Pulse 120, respiration 78, salivating, seems excitable, defecated, bad control of movements.

3.30 Pulse 120, respiration 55, bad control of movements, diarrhea.

3.45 Pulse 60, respiration 84, moves around fairly well.

4.00 Movements unsteady and spasmodic, convulsions begin.

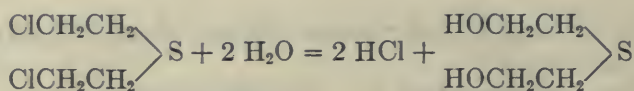
4.05 Unable to stand.

5.00 Respiration 72, lying prone in convulsions.

March 30. Found dead.

Autopsy. *Trachea* normal; *lungs*, one lobe appears congested; *duodenum*, deeply blood stained contents; *intestines* congested with blood stained contents; *heart*, few small subendocardial hemorrhages.

The injection of saturated aqueous solutions which have been allowed to come to room temperature or which have been standing for any time, show much diminished effects, or, if several hours' old, are entirely without effects. This was tried by intravenous injection and also on the eye and skin. Such solutions are known to be partially or completely hydrolyzed into hydrochloric acid and dihydroxyethylsulphide.



Experiment 13. Dog V11; weight, 4 kilos.

March 22.

3.00-3.15 Injection intravenously of 160 cc. of aqueous solution twenty-four hours old (40 cc. per kilo).

4.00 No effects.

6.00 No effects.

March 23.

9.00 Dog perfectly normal. No vomiting or diarrhea during night.

Experiment 14. Dog EM 970; weight, 8 kilos.

2.40 Pulse 130, respiration 18.

2.50 Intravenous injection of 10 cc. per kilo of a saturated, aqueous solution of dichlorethylsulphide which had stood for three and one-half hours.

3.00 Pulse 160, respiration 18.

3.50 Pulse 160, respiration 22, dog seems normal.

4.15 Pulse 164, respiration 20. Animal appeared perfectly normal while under observation for two or three days.

Experiment 15. The cold, fresh, aqueous solution was applied to the forearms of five men. After standing at room temperature for two hours it was again applied. Twenty-four hours later, four out of five of the men showed a distinct reaction from the fresh solution, none exhibited any effects from the solution two hours old.

2. *Effects of inhalation of large doses of the vapor.* Some of the effects which have been observed from the injection occur when a dog is poisoned by inhalation of the vapor. When a dog is poisoned by inhalation of a very large dose of the vapor, practically all the effects obtained by injection—salivation, vomiting, bloody diarrhea, hyperexcitability and convulsions, slow, irregular pulse, becoming rapid before death, and paralysis of the vagi—all have been observed in dogs poisoned by inhalation. This leaves little room for doubt that in high concentrations, dichlorethylsulphide is absorbed through the lungs and produces its characteristic effects upon the body.

Experiment 16. Dog V12, male; weight, 11.4 kilos.

March 21.

10.15 Pulse 168, respiration 36. Very active and playful.

10.30–11.30 In gassing chamber, exposed to 0.3 mgm. per liter of dichlorethylsulphide—restless, rapid respiration, excitement, salivation, vomiting.

11.40 Hyperexcitable. Coughing.

11.50 Pulse 108, respiration 56.

1.00 Defecates. Marked muscular weakness, ataxia.

1.25 Convulsions.

1.45 Pulse 152, respiration 50.

3.30 Pulse 168, respiration 64, marked muscular weakness, salivation.

4.00 Vomits.

4.30 Pulse 176, respiration 52, pulse becoming feeble.

Killed with ether.

Autopsy. Mouth, esophagus, and stomach normal; duodenum shows congestion, contents bloody; small intestine has congested areas of mucosa; trachea, some slight membrane; lungs, one lobe congested and emphysematous; heart, kidneys, liver, and spleen, normal.

Experiment 17. Dog V13, female; weight, 13.5 kilos.

10.00 Pulse 96, respiration 26.

10.05–11.05 In gassing chamber, exposed to 0.28 mgm. per liter of dichlorethylsulphide. Excitement, irritation of upper respiratory tract, salivation, and vomiting.

11.08 Loose stools.

11.35 Pulse 44, respiration 96, pulse irregular, expiration spasmodic.

12.35 Pulse 44, respiration 60, diarrhea, salivation, hyperexcitability with typical convulsions.

1.20 More violent convulsions.

2.05 Pulse 68, respiration 28. Animal very weak.

2.30 Vomits voluminous, foamy fluid. Bloody diarrhea.

3.40 Pulse 70, respiration 30. Very weak, but still struggles some.

4.00 Killed with ether.

Autopsy. Trachea slightly congested, contains some fluid; lungs, one lobe shows slight hemorrhage, few patches of emphysema; esophagus and stomach normal; upper intestinal tract deeply congested, congestion decreases until in ileum, normal; large intestine congested.

3. *Fate of dichlorethylsulphide in body.* Further convincing proof of the absorption of the sulphide through the lungs is furnished by the detection of one of the products of hydrolysis, dihydroxyethylsulphide in the urine of animals poisoned by dichlorethylsulphide by inhalation. This hydrolytic product can also be found in the urine after the injection of mustard gas.²

Dihydroxyethylsulphide was injected into a dog, the urine collected and examined for this substance. A positive test was obtained. Urine from a normal dog failed to give a positive reaction. This was necessary because of the occurrence of ethylsulphide and its precursors in normal dog's urine. This proves that the substance is excreted, in part at least, unchanged. The injection of dichlorethylsulphide was next tried, and the urine found to contain the dihydroxyethylsulphide. The inhalation experiments were then performed.

Experiment 18. Dog V47, female; weight, 13.2 kilos.

May 19. Intravenous injection of 100 mgm. per kilo of dihydroxyethylsulphide in 10 cc. saline.

May 20. No urine passed in cage. 230 cc. urine by catheter. This was evaporated under diminished pressure to small volume, 25 cc. of concentrated hydrochloric acid was added, and distillation carried out under diminished pressure. The distillate was extracted with ether, and the extract evaporated. The residue was tested by applying small amounts to the skin of several individuals. A typical mustard gas reaction developed. This was considered evidence of the presence of dichlorethylsulphide.

Experiment 19. Dog V49, female; weight, 21.4 kilos.

May 20-21. Over a period of twenty-four hours, injected subcutaneously with 60 mgm. per kilo of dichlorethylsulphide in olive oil, 10 mgm. per kilo at a time. Immediately after last injection, dog was catheterized and 200 cc. urine obtained. This was concentrated as above, and tested for dichlorethylsulphide by applying to the skin of several men. No reaction obtained. It was then treated as above, and a slight amount of oily substance obtained on the distillate. The

² We wish to thank Mr. D. J. Beaver for aid in the work on the detection of this compound in the urine.

distillate was extracted with ether, and the ether evaporated, leaving a globule of oil. This oil was placed in a small test-tube and the mouth of the tube held against the arm for three minutes. After twenty-four hours a distinct dichlorethylsulphide effect was obtained. The distillate contained mustard gas, and the urine dihydroxyethylsulphide.

Experiment 20. Dog V54.

May 24

10.25-10.55 Gassed in continuous flow chamber for 30 minutes, 0.52 mgm. per liter.

May 25.

9.00 Pulse 102, respiration 34. Great muscular weakness. Unobserved, but seems to have been in convulsions.

10.00 Urine taken with catheter and examined for dihydroxyethylsulphide.

10.07 Dog dies in coma.

A severe reaction from the re-chlorinated product was not obtained, but a sufficient reaction was obtained to furnish evidence of dichlorethylsulphide being present.

It was found possible by the application of 0.1 to 0.2 gram per kilo to the skin of dogs to obtain the characteristic effects of the absorption of mustard gas: Salivation; vomiting; diarrhea; hyperexcitability; rapid, feeble pulse and depression, but neither convulsions nor slowing of the heart. The product of hydrolysis of mustard gas was also detected in the urine.

Experiment 21. Dog V90; weight, 16 kilos.

11.00 Normal pulse 108, respiration 36. Chest and abdomen shaved. 1.6 grams of pure dichlorethylsulphide rubbed into skin with glass rod. Animal placed so that draft prevented absorption by inhalation of the vapors.

11.30 Second application; 1.6 grams rubbed in. (Total quantity, about 200 mgm. per kilo).

12.00 Pulse 96, respiration 150.

1.40 Pulse 138, respiration panting.

5.00 Pulse 168, respiration panting. Dog placed in clean metabolism cage.

June 4.

9.00 200 cc. of urine collected over night; examined for dihydroxyethylsulphide. Positive findings. Pulse 198, respiration 36
Dog depressed, slight salivation.

June 5.

9.00 Pulse 186, respiration 48. Dog etherized.

III. MECHANISM OF ACTION

1. *Toxicity of products of hydrolysis.* The latent period in the development of the effects of dichlorethylsulphide, either local upon the eyes, skin or respiratory tract or systemic upon the heart, nervous system and digestive tracts, suggests that the substance may be altered in the body before exhibiting its characteristic actions. In fact, the absence of any immediate effects when the aqueous solution is injected directly into the blood stream makes this assumption almost imperative. The simplest chemical change which this substance undergoes *in vitro*, is hydrolysis into hydrochloric and dihydroxyethylsulphide. That this change takes place in the animal organism is shown by the detection of dihydroxyethylsulphide in the urine. However, an injection of a hydrolyzed solution of dichlorethylsulphide is without effect. The dihydroxyethylsulphide, when applied pure to skin of man and dogs, produces no irritation, whatever (5). As much as 0.3 gram per kilo was injected intravenously into a dog without producing any apparent effect, immediate or remote. An injection of 1400 mgm. per kilo, caused only a slight stupor and loss of co-ordination, with a quick return to normal, and none of the symptoms of the dichlorethylsulphide were present.

Experiment 22. Dog EM575 male; weight, 7.8 kilos.

November 18.

2.30 Normal pulse 80, respiration 20.

2.45 Intravenous injection of 1400 mgm. per kilo of dihydroxyethylsulphide in 100 cc. of water (total 10.920 grams). Injection followed by salivation and slight nausea.

2.50 Dog shows slight ataxia. Pulse 120, respiration 20.

3.10 Pulse 110, respiration 20.

3.30 Pulse 110, respiration 20. Dog appears stupid, and exhibits a slight ataxia similar to light alcohol poisoning. No serious symptoms apparent.

5.00 Pulse 105, respiration 20. Dog quiet, perhaps slightly depressed. Eats and drinks with indifference.

November 19.

9.00 Dog normal. Pulse 98, respiration 24.

November 20.

9.00 Dog normal. Pulse 105, respiration 30.

Experiment 23. Dog EM576. Weight 5.9 kilos.

November 19.

10.30 Normal pulse 100, respiration 20.

10.45 Intravenous injection of 200 mgm. per kilo, (total 1200 mgm.) dihydroxyethylsulphide in 50 cc. of water. Animal exhibited no symptoms, whatever. Observed for three days.

Experiment 24. The pure dihydroxysulphide was rubbed into the skin on four dogs and five men, and produced no irritation, either immediate or remote.

This product of hydrolysis is not responsible for the effects of mustard gas. The other product of hydrolysis is hydrochloric acid and is not a very toxic substance. Relatively large amounts can be injected intravenously without producing any marked effect. This is readily understood. When injected intravenously it is immediately neutralized by the buffer action of the blood. The blood does not become acid and the tissues are never really exposed to the acid. When strong solutions are placed on the skin or mucous surfaces, or injected into the tissues, an irritating effect is noticed. Hydrochloric acid, however, injected in very large doses, does produce very definite effects upon the animal and can cause death. Both products of hydrolysis of mustard gas are very readily soluble in water and very sparingly soluble in organic solvents, or, in other words, have a low lipid solubility or partition coefficient. It would be expected from this that they would not readily penetrate cells. Harvey has shown this to be true for hydrochloric acid (6).

2. *Theory of action.* Dichlorethylsulphide is very slightly soluble in water and very freely soluble in organic solvents, or has a high lipid solubility or partition coefficient.³ It would, therefore, be expected to penetrate cells very readily. Its rapid powers of penetration are practically proven by its effects upon the skin. Having penetrated within the living cell, it would undoubtedly hydrolyze. The liberation of free hydrochloric acid *within the cell* would produce serious effects and might account for the actions of dichlorethylsulphide. To summarize, then, the mechanism of the action of dichlorethylsulphide appears to be as follows:

1. Rapid penetration of the substance into the cell by virtue of its high lipid solubility.

2. Hydrolysis by the water within the cell, to form hydrochloric acid and dihydroxyethylsulphide.

3. The destructive effect of hydrochloric acid upon some part or mechanism of the cell.

Although hydrochloric acid does not penetrate cells readily and is easily neutralized by the buffer action of the fluids of the body, we might expect by flooding the body with large quantities of acid to produce some of the characteristic effects of mustard gas. Stimulation of the respiratory center is a well known effect of acid. Convulsions and salivation may be produced by injection of hydrochloric acid and we have been able to produce slowing of the heart by rapid injection of this acid.

The delayed action of mustard gas might be explained by the formation of some compound with some constituent of the blood. However, blood taken from dogs which had been poisoned with mustard gas and were exhibiting typical symptoms at the time injected into normal dogs, produced no effect. Serum treated *in vitro* with mustard gas and allowed to stand and then injected into a dog, produced no effect. The fluid which is formed in the vesicles and blebs produced by the application of mustard gas to the skin produces no mustard gas effects.

³ Attempts to determine accurately the partition coefficient of this substance are unsuccessful, due to the rapidity with which it hydrolyzes. It appears to be over 200, using xylene and water at 20°C.

3. *Effects of administration of sodium bicarbonate.* By the administration of large quantities of sodium bicarbonate both intravenously and by mouth, the symptoms following an intravenous injection of dichlorethylsulphide can be delayed and convulsions, but not death, can be prevented.

Ten cubic centimeters per kilo of the fresh, aqueous mustard gas solution given intravenously always causes convulsions, violent other symptoms and death. A series of 11 dogs injected with this dose of the solution, were treated with 10 cc. per kilo of 5 per cent sodium bicarbonate by mouth and intravenously every hour for five or six hours. In one of these animals no benefit was obtained, in 6, symptoms were delayed and milder, in 4, convulsions were prevented. Death was never prevented, although some times apparently delayed. The action of mustard gas on the heart appeared to be increased, and most of the animals showed a very slow, failing heart before death. Since sodium bicarbonate (Harvey) is known to penetrate cells only with difficulty, much benefit could not be expected. Numerous amines, especially those of high lipid solubility, have been tried, but thus far the effects have not been consistent. A substance possessing the same physical properties as mustard gas, but slowly yielding alkali on hydrolysis, would be ideal to try for treatment.

4. *Effect of temperature on toxicity.* The fact that the velocity of hydrolysis of dichlorethylsulphide is very much decreased by lowering the temperature, suggested trying the effects on animals at a high and low temperature. The influence of temperature is quite marked on drugs which undergo a change in the body before acting. The lethal dose of both atoxyl and colchicin is increased markedly for frogs when the temperature is lowered, and decreased when it is raised to 37°C. (7). Atoxyl is supposed to be reduced in the body before acting and colchicin is oxidized. According to the theory which has been advanced for the mode of action, one might expect that at a low temperature the rate of liberation of hydrochloric acid in the cell might be slow enough to be non-toxic, for a dose which would prove fatal at a higher temperature.

Fish were used for our experiments. Those kept at a low temperature survived the same dosage which proved fatal to those kept at room temperature.

Experiment 25. Seventeen healthy catfish were exposed in groups of four or six, to dichlorethylsulphide by immersing in a quarter saturated solution of mustard gas in tap water at 10° for five minutes. Ten of these were placed in water at room temperature (24° to 26°), and ten died (100 per cent) within twenty-eight hours. The other seven fish were kept at 8° to 10° , and one died in ninety-six and another in one hundred and eight hours, while the other five survived for five days, when observations were discontinued.

Experiment 26. Fifteen goldfish were exposed as in experiment 25 to a half-saturated solution for ten minutes. Eight were transferred to a bath at room temperature (25°) and seven to a bath at 8° to 10°C . Of the first group, four died in twenty-six hours, two died in fifty-six hours, and two died in six days. Of the second group, all survived seventeen days, when observations were discontinued.

It was quite noticeable that in the catfish which were kept at room temperature hemorrhages generally developed in the fins and tails and in the ventral surface of the body after sixteen to twenty hours,⁴ while in those kept cold, no hemorrhage was ever observed. Symptoms, which were marked in the first group, were never observed in the second.

Although this evidence fits in perfectly with our theory, it is well known that the effect of drugs is changed by decreasing temperature (8). The following experiments indicate that hydrochloric acid is as toxic for fish whether the fish are cooled after exposure or kept at room temperature.

Experiment 27. Eight catfish were exposed to 0.05 per cent hydrochloric acid for five minutes. Four were placed at room temperature (25°C .) and four placed at 8° to 10°C . Of the first group, all died within forty minutes; of the second, three died within thirty minutes, and 1 in one hundred and fifty minutes.

⁴ It is interesting to note that dichlorethylsulphide appears to act on the skin of catfish and not that of goldfish.

Experiment 28. Sixteen goldfish were exposed in the same manner as in experiment 27. Eight were kept at room temperature (20°C.), and eight at 8° to 10°C. Of the first group two died in one hour, and six in one hour and 20 minutes. Of the second group, all died within one hour.

An experiment on atropine and one on sodium cyanide indicated that these substances were just as toxic for fish whether they were kept at ordinary temperature or cooled, after exposure.

Experiment 29. Four goldfish were exposed for ten minutes to a 0.01 per cent atropine sulphate solution. Two were placed in a bath at 20°C. and two in a bath at 8° to 10°C. In the first group, both died in six to seven hours; in the second group, both died in five to seven hours.

Experiment 30. Six goldfish were exposed for ten minutes to 0.48 per cent solution of sodium cyanide. Three were kept at 20°C. and three at 8° to 10°C. They died in three, five and eight hours, and four, six and eight hours respectively.

Catfish survive fifteen times the concentration of hydrochloric acid present at the end of five minutes in the half-saturated water solution of mustard gas, and three times the concentration present at the end of twenty-four hours. Fish will survive fifteen minutes' exposure in this solution after it has stood one hour at room temperature.

It is evident that these experiments on fish tend to substantiate the theory of intracellular liberation of acid.

5. *Properties and action of some compounds related to dichloroethylsulphide.* Various compounds related to mustard gas have been prepared at one time or another by the Offense Chemical Section of this Station. A cursory survey of their lipid solubility, rates of hydrolysis, and pharmacological effects, have been made.

The lipid solubility was estimated by using xylene and water. Victor Meyer (9) noted that ethylsulphide was inactive, while the β -monochlorethylsulphide was less active than mustard gas. Monochlorethylsulphide and the two isomeric β - β -dichlor-

propylsulphides, are all highly (though in different degree) lipid soluble and all hydrolyze more or less rapidly in aqueous solution. All are active skin irritants, and more or less toxic on inhalation, producing comparable lesions and symptoms to mustard gas.

6. *Discussion.* The theory of the intracellular liberation of hydrochloric acid as the mechanism of action of dichlorethylsulphide, explains all the experimental facts thus far observed. The histological changes in the skin have been stated to resemble hydrochloric acid burns. "The lesion is a chemical burn unlike that produced by heat, electricity, or the ordinary corrosives such as sulphuric, nitric, and hydrochloric acids, or strong alkalies. Of all these agents, the effects are most closely allied to those of hydrochloric acid, but are much greater in intensity" (10). Lillie, Clowes, and Chambers (11), in a study of the action of mustard gas on marine organisms especially starfish eggs, have obtained evidence which supports our interpretation of the action of dichlorethylsulphide, e.g., an intracellular liberation of hydrochloric acid as the toxic factor.

A theory to explain the "edema, fat infiltration, multiple hemorrhages, and necrosis of the central portion of the liver lobule" by the "severe tissue effects by the halogen acids formed in the tissues" has been advanced by Graham (12) for the delayed poisoning by chloroform and other alkyl halides. Graham's evidence is briefly as follows: Similar morphological changes can be produced especially in the liver by injection of hydrochloric acid into the portal vein. Sections of the chloroformed liver show an acid reaction with neutral red or Nile blue immediately, whereas such changes occur more slowly in the liver of normal animals after death. Carbon tetrachloride is more effective than chloroform and methylene chloride less effective in producing liver necrosis: this is in the same order as the amount of hydrochloric acid that can be liberated. Simultaneous injection of sodium carbonate with the chloroform anaesthesia prevents or decreases the liver injury and other effects. Other alkyl halides give a typical morphological picture of chloroform poisoning. That these substances form halogen acids is shown by the excretion of the neutral salts in the urine.

Chloral, which does not yield an appreciable quantity of hydrochloric acid in metabolism, does not produce these changes. Furthermore, he states that "the question of how the acid is formed will not be discussed in this paper." He, however, suggests Nef's ideas of dissociation, forming bivalent carbon.

It is evident that the theory suggested in this paper is somewhat different. Graham does not consider intracellular liberation of acid, and is dealing with substances which do not readily yield hydrochloric acid on contact with water, but must undergo relatively slower metabolic changes by the tissues. The effects of mustard gas are not similar to those of delayed chloroform poisoning and, moreover, chloroform is a substance of an entirely different order of toxicity.

It is not impossible to reconcile these two different types of poisoning as both being due to hydrochloric acid. Graham's suggestion that the "liver is an organ which most strikingly manifests the chloroform necrosis, and that this organ is also the site of a most active metabolism, harmonize well with this hydrochloric acid theory" may explain the difference. As stated above, chloroform must be slowly metabolized to produce hydrochloric acid. By oxidation chloroform yields phosgene and hydrochloric acid. Phosgene readily yields hydrochloric acid on hydrolysis.

We are inclined to agree with Graham when he states that "Particular care has been exercised all through this article to state that the halogen acids are suggested to be important factors rather than the only factor involved. Other acids must play a part, and possibly other substances than acids, are involved."

We feel in the case of mustard gas and analogous compounds where the radical other than the acid radicle is non-toxic, that the entire responsibility can be laid to intracellular production of acid. The question of cell penetration as well as ease of hydrolysis and lipid solubility, has to be considered in dealing with a series of compounds.

It is interesting to note that most of the war gases can readily yield a halogen acid by hydrolysis. Whether the intracellular

liberation of acid will explain their relative toxicity, must be decided by future work. Their partition coefficients, their rate of hydrolysis, their volatility and other physical chemical properties, may explain the differences in localization and intensity of action. The facts which can be gathered from the literature concerning dimethylsulphate, seem to indicate that its toxic action may be due to the intracellular liberation of sulphuric acid. The symptoms recorded in the literature due to this poison, are very suggestive of mustard gas poisoning—local redness and edema, generalized toxic and clonic convulsions, coma and death (13). The effect on the eyes (14) and the clinical descriptions of several factory cases of poisoning resemble mustard gas (15). Both Weber and Michiels (13), who worked experimentally on animals with this substance, discuss the possibility of the effects being due to acid poisoning. They decide against it because the amount of acid liberated is too small, the blood gases of the poisoned animal were found normal, sodium carbonate did not help poisoned animals, and it is doubtful whether time elapsed for a toxic dose to hydrolyze. Michiels found, moreover, that serum treated with a toxic dose of dimethylsulphate, incubated and injected into a rabbit, was non-toxic. So he agrees with Weber, that the molecule is toxic and not a decomposition product. The evidence appears to us to be very suggestive for an *intracellular* liberation of sulphuric acid as the toxic factor. We have found the partition coefficient to be about 1.9, using xylene and water at 20°C. and that this substance hydrolyzes much more slowly than dichlorethylsulphide. It would be expected that the toxicity on intravenous injection would be less than mustard gas. Preliminary experiments indicate that 50 mgm. per kilo are necessary to produce the same symptoms as are given by 6 mgm. per kilo of the dichlorethylsulphide. The symptoms appeared much more quickly in the case of dimethylsulphate. This would indicate that mustard gas is prevented for a time from hydrolysis by being held in lipoids, while dimethylsulphate, having a much lower partition coefficient, is more readily transferred to an aqueous phase for hydrolysis. Certain facts which we have observed in

the case of the action of dichlorethylsulphide on the skin also led to this conclusion (16).

IV. SUMMARY

1. Dichlorethylsulphide is absorbed through the lungs and produces definite, characteristic, systemic effects.

2. The symptoms of injection of the substance are salivation, vomiting and diarrhea, tonic and clonic convulsions, slow and irregular heart, followed by a rapid pulse, and stimulation of the respiration.

3. A dose of six milligrams or less per kilo, injected intravenously in aqueous solution, proves fatal for dogs.

4. Dichlorethylsulphide appears to be excreted in the urine, in part at least, as dihydroxyethylsulphide, which has been shown to be a comparatively non-toxic body.

5. The lesions in the intestine suggest that excretion of the substance may also take place here.

6. A theory of action has been advanced. The dichlorethylsulphide penetrates the cells, and in the aqueous phase of the cell, hydrolyzes to hydrochloric acid which is responsible for the damage.

7. Sodium bicarbonate in large doses somewhat alleviates the symptoms, but does not prevent death.

8. Fish are much less susceptible to this substance when kept at a low temperature after exposure rather than at room temperature. The hydrolysis of the substance is much slower at a low temperature.

9. Monochlorethylsulphide, and dichlorpropylsulphide are lipid soluble and easily hydrolyzed. They both act similarly to mustard gas.

10. Graham's hydrochloric acid theory of chloroform poisoning has been discussed, and the application of these ideas to general problems of toxicity.

11. A review of the literature on dimethylsulphate and a few observations of our own would lead us to believe that this substance acts by intracellular liberation of sulphuric acid.

REFERENCES

- (1) An immense amount of work has been done on dichlorethylsulphide both in this country and abroad. A great deal of this is in the form of confidential reports which cannot at present be referred to. Among the articles on this subject which have recently appeared in Medical Journals, are:
 MANDEL, M., AND GIBSON, W. S.: Jour. Am. Med. Ass., 1917, lxix, 1970.
 WARTHIN, A. S., AND WELLER, C. V.: Jour. Lab. Clin. Med., 1918, iii, 447.
 DERBY, G. S.: Am. Jour. Med. Sciences, 1918, clvi, 733.
 WARTHIN, A. S., WELLER, C. V., AND HERRMANN, G. R.: Jour. Lab. Clin. Med., 1918, iv, 787.
 GIRAUD, A.: Jour. de Med. et de Chir. Prat., 1917, lxxxviii, 890.
 TEULIERES: Jour. de Med. de Bordeaux, 1917, No. 12, 247.
 PISSARELLO: Gior. di Med. Mil., 1918, lxvii, 128.
 CORTINI: Gior. di Med. Mil., 1918, lxvi, 235.
 CANELLI: Riv. Ospedal., 1918, viii, 2.
- (2) MEYER, V.: Ueber Thiodiglykolverbindungen. Berichte, 1886, xix, 3259.
- (3) The effects from inhalation will be reported in detail in a subsequent communication.
- (4) Unpublished observations.
- (5) VICTOR MEYER states that this substance is non-toxic. Berichte, 1887, xix, 3259.
- (6) HARVEY, E. N.: The permeability of cells for acids. Publication No. 212 of Carnegie Institution of Washington, 1915, p. 143.
- (7) SANNO, Y.: Über den Einfluss der Temperatur auf die Giftempfindlichkeit des Frosches. Arch. f. Exp. Path. u. Pharm., 1911, lxv, 325.
- (8) According to SOLLMANN (Manual of Pharmacology, 1917, p. 94), "Digitalis, veratrin, nicotin, strychnin, tetanus toxin, chloral and alcohol are rendered more active, morphin and curare, less active, by raising the temperature" for cold-blooded animals.
- (9) MEYER, V.: Medicinische-chemische Notizen. Berichte, 1887, xx, 1729.
- (10) WARTHIN, A. S., AND WELLER, C. V.: The pathology of the skin lesions produced by mustard gas. Jour. Lab. Clin. Med., 1918, iii, 478.
- (11) Personal communication from Dr. G. H. A. Clowes.
- (12) GRAHAM, E. A.: Late poisoning with chloroform and other alkyl halides in relationship to the halogen acids formed by their chemical dissociation. Jour. Exp. Med., 1915, xxii, 48.
- (13) WEBER, S.: Ueber die Giftigkeit des Schwefelsäure dimethylesters und einigen Verwandten Ester der Fettreihe. Arch. f. Exp. Path. u. Pharm., 1902, xlvii, 113.
- MICHIELS, J.: Sur la toxicité du sulfate neutre de methyle. Arch. Int. de Pharm. et de Ther., 1911, xxi, 467.
- (14) ERDMANN, P.: Über Augenveränderungen durch Dimethylsulfat. Arch. f. Augenheilk., 1908-09, lxii, 178.
- (15) WEBER, S.: loc. cit.
- (16) See following paper.

ON DICHLORETHYLSULPHIDE (MUSTARD GAS)

II. VARIATIONS IN SUSCEPTIBILITY OF THE SKIN TO DICHLORETHYLSULPHIDE

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Every investigator who has worked with dichlorethylsulphide has noticed that some individuals are much more susceptible to skin burns from this substance than are others. Victor Meyer (1) noted that his assistant was greatly affected in working with the substance while he himself was not. Similar observations have been made by many workers in the laboratory. In the course of work in this laboratory it has been possible to demonstrate beyond a doubt a great individual variation in susceptibility of the skin, and to devise methods for the determination of the sensitivity of an individual.

Methods. Two methods have been used for determining the cutaneous sensitivity of individuals to dichlorethylsulphide. The first of these methods consists in exposing skin to the vapors of the substance under constant conditions and determining the minimum time of exposure which is necessary to produce a visible reaction within twenty-four hours. The apparatus which is used in this method is shown in the accompanying illustration (Fig. 1). It consists of a small test tube (1 cm. by 10 cm.) containing a cotton plug saturated with pure dichlorethylsulphide held by means of a rubber stopper in a larger test tube which is filled with water. When not in use the smaller tube is closed with a cork stopper. On being first prepared the tubes are allowed to stand unstoppered for about twenty-four hours in

order to remove any volatile impurities that may be present. Before use the whole apparatus is placed in a constant temperature water bath. In all this work 20°C . has been taken as a constant temperature for making exposures. The skin is exposed to the vapors in the smaller tube by holding the mouth of the tube firmly against the skin. Exposures are made for different lengths of time in order to determine the shortest exposure which will just produce a visible reaction, the minimum burning time. The reaction is not visible for some hours and

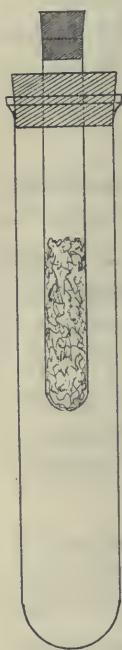


FIG. 1

is noted twenty-four hours later. The reaction consists in a circular area of erythema which is usually uniform. In certain cases a group of small, red spots may be all that is noticed. Longer exposure than the minimal will give rise to edema as well as erythema, and a still longer one, to subsequent small vesicles or a well defined blister over the entire area exposed (2). In this method the skin is exposed to supposedly saturated vapor at 20°C .

The second method which has been used for studying the sensitivity of individuals consists in applying to the skin standard solutions of mustard gas in paraffin oil. For most purposes the best solutions are a 1 per cent (1 gram of pure dichloroethyl-sulphide in 100 cc. of oil), a 0.1 per cent and 0.01 per cent. A small drop of each of these solutions is applied to the skin of the forearm and the arm allowed to remain uncovered for about ten minutes. The presence or absence of a positive reaction is indicated by the appearance or absence of erythema

twenty-four hours later. Other solvents can be used but the reactions are only comparable when the same solvent is used in making all tests. Linseed oil, cottonseed oil, kerosene and alcohol have been tried. The solution in absolute alcohol is the most reactive, while those in linseed, cottonseed and kerosene oils appear to be somewhat less reactive; that is a more dilute solution in alcohol will give a positive reaction in any given individual than one in paraffin oil. The fresh, saturated, aqueous solution

(0.07 per cent) will give a reaction on the skin of a sensitive individual and one man was found who reacted to one drop of a 1:100 dilution, that is; to about 0.0000005 gram of the substance. Within certain limits the amount of a given solution in paraffin oil which is applied makes no difference in determining whether the reaction is positive or negative. It makes a difference in the intensity of the burn. A large amount of the 1 per cent solution may cause a blister while one-tenth the quantity will not. We have used a glass rod dipped in the solution and then touched to the skin after the excess had been shaken off, and also a pipette or an ordinary marking pen to apply the solutions. Removing the excess of the solution five minutes after the application appears to make no difference in the result. All our subjects were instructed to leave the sleeve rolled up for ten or fifteen minutes after the test was applied. Twenty-four hours after making the test, results are recorded. Readings made forty-eight hours later gave the same result. Less than twenty-four readings should not be taken because some individuals are rather slow in reacting.

The first method undoubtedly gives the most accurate results, although the second is very much simpler and more rapid in its application. A number of tests have been made on individuals by both methods in order to determine if each method gives the same result. Men who are sensitive to one test are sensitive to the other, and vice versa. Roughly, it may be said that a man who gives a reaction to the 0.01 per cent solution will show a reaction from a fifteen second (or less) vapor exposure, while a man who shows no reaction to the 1 per cent solution will be negative or only faintly positive to a four minute vapor exposure.

Results of vapor method. This method has only been applied to a few individuals working around the laboratory. The results are shown in the table 1.

It is interesting to note that the very extreme cases have a minimum burning time of one second and ten minutes respectively. In other words, we can roughly say that one of these individuals is 600 times as sensitive as the other. The man who gave a reaction from a one-second exposure blistered from a

five second exposure or one to which the vast majority of individuals will give no reaction. When first found, this subject blistered from the application of the 0.01 per cent solution in paraffin oil. Fortunately we have been able to obtain a complete history and physical examination, but nothing of note was found. It can be stated positively that this man had never been exposed to mustard gas nor had he received even a small experimental burn when first tested.

TABLE 1

Time of exposure to vapor tests to produce a visible reaction

SUBJECT	TIME	SUBJECT	TIME	SUBJECT	TIME
	<i>seconds</i>		<i>minutes</i>		<i>minutes</i>
H. C. J.....	1	V. L.....	1	— R.....	4
J. A.....	5	— F.....	1	R. H.....	4
H. P.....	5	H. W.....	1	— G.....	4
L. D. S.....	5	— R.....	1	C. I. R.....	4
W. V. C.....	10	L. S.....	1	— P.....	4
H. W. S.....	15	— C.....	1	P. J. H.....	4
E. K. M.....	15	F. W. F.....	1	F. W. S.....	5
J. W. W.....	15	— W.....	1	G. L. S.....	5
C. L. H.....	15	La P.....	1	R. A. T.....	5
C. H.....	15	— K.....	2	— B.....	5
— H.....	30	— Y.....	2	— K.....	7
— N.....	30	— L.....	2	M. R. T.....	10
R. E. W.....	30	G. A. D.....	2	— B. (colored)....	10
— P.....	30	— L.....	3	C. B. (colored)....	10
— A.....	30	— D.....	3	C. W. B. (colored) .	10
A. W. K.....	30	— S.....	3	O. H. (colored)....	10
A. C. S.....	30	H. L. I.....	3	H. B. (colored)....	10
H. H. C.....	30	C. B. M.....	3	W. M. L. (colored) .	10
		C. F. W.....	3		

Results of tests with oil solution. As has been pointed out, the oil solution method of determining sensitivity is very much easier and more rapid in its application than the vapor test method. Results have been obtained on rather large groups of individuals, using this method. Table 2 summarizes the results obtained on a group of men at this Station. Practically all these men were indoor workers, and a great many of them had been more or less exposed to mustard gas.

A similar test on a larger scale was carried out at the Edgewood Arsenal, Edgewood, Maryland. Very few of the men tested (twenty) had ever been exposed to mustard gas, and most of the men were outdoor workers. In this case only the

TABLE 2

REACTION			NUMBER OF MEN	PERCENTAGE OF TOTAL
1 per cent	0.1 per cent	0.01 per cent		
Positive	Positive	Positive	7	2.0
Positive	Positive	Negative	26	7.5
Positive	Negative	Negative	238	68.6
Negative	Negative	Negative	76	21.9
Total.....			347	100.0

TABLE 3

REACTION		NUMBER OF MEN	PERCENTAGE OF TOTAL
1 per cent	0.01 per cent		
Positive	Positive	43	3.3
Positive	Negative	709	55.3
Negative	Negative	530	41.4
Total.....		1282	100.0

TABLE 4

REACTION		NUMBER OF MEN	PERCENTAGE OF TOTAL
1 per cent	0.1 per cent		
Positive	Positive	0	0.0
Positive	Negative	13	15.0
Negative	Negative	65	78.0
Questionable	Negative	6	7.0
Total.....		84	100.0

1 per cent and 0.01 per cent solutions were used. Table 3 summarizes the result.

Table 4 is the result of a test made on negroes. The few of these previously examined by the vapor test had been found very resistant. Only the 1 per cent and 0.1 per cent solutions were used.

It is seen from the above tables that negroes as a race, have a much more resistant skin than white men. No negro of the 84 examined reacted to the 0.1 per cent solution, and of course none would react to a more dilute one. About 10 per cent of white men react to the 0.1 per cent solution, while 2 to 3 per cent react to the 0.01 per cent solution or are hypersensitive. About 78 per cent of the negroes fail to react to the 1 per cent solution, while only 20 to 40 per cent of the white race do not show a reaction.

Variation in susceptibility in the same individual. In the above discussion it has been tacitly assumed that the sensitivity of the same individual is always the same. Such is not the case. Extended series of experiments have been carried out to determine what conditions influence the sensitiveness of an individual, and under as near comparable conditions as possible, the change in susceptibility from day to day in a group of individuals.

The moist parts of the body have been stated to be more susceptible to burns than other portions (3). The effect of exercise and sweating was investigated. A number of individuals were given vapor burns (one to five minutes exposure) and then exercised until in a profuse sweat, and then the same exposure to vapors, made. In all cases the burn produced after exercising was more severe. Sweating produced by having the subjects place their feet in hot water, produced the same increase in susceptibility. That the moisture on the skin produced by sweating is at least partly, if not entirely, responsible for the increased susceptibility, was shown in the following way: An area of the forearm was kept moist for a few minutes with wet cotton. The sponge was then removed and two vapor tests made, one over the moist area and one over normal, dry skin. In all cases the moist burn was the more severe, in one, producing a blister where the control did not.

The skin of different areas of the body is undoubtedly somewhat different in its susceptibility. All our tests have been applied to the forearm. The hands are considerably more resistant than the forearm. Tests made by the oil method on the forearm, chest, and back, however, indicate very little dif-

ference in susceptibility of these areas. The skin in the neighborhood of old burns has been shown to be more susceptible.

It is the general impression among workers in mustard gas that they became more susceptible to skin burns from continued exposure to the vapor. A series of tests for skin sensitivity (oil method, using 1, 0.1, and 0.01, per cent solutions) over a period of two months on eighteen men working in a small scale development mustard plant failed to demonstrate any more change in the reaction of these men than in a similar group of men working in the laboratory.¹ Of course, the change may have been too small to detect with the method. In the case of one of the authors (H. W. S.) there is proof of a definite increase in sensitivity with the development of an atypical reaction. The following is a brief description of the case:

This subject has been working in the laboratory with mustard gas on and off for ten months, and for the last six months, daily. No serious effects have ever been experienced. No accidental burns of any magnitude have ever been received, and the only effects have been occasional laryngitis and conjunctivitis. When our sensitivity test was first devised (May, 1918) this subject had a minimum burning time of two minutes. Repeated, small, superficial, experimental burns have been on this subject both before and after this time. The burns always ran the usual course—appearance of erythema in one and one-half to two hours, edema in twelve to eighteen hours, followed by vesication, and sluggish healing. On October 10, 1918, no experimental burns had been made on the subject for a period of two or three weeks. On this date, a small experimental burn with the pure liquid sulphide was made on the left wrist. Six minutes after the application the subject experienced severe itching, and on observing the affected area, noticed a distinct erythema which, in a few minutes, progressed to a well defined wheal. The reaction persisted for forty to sixty minutes, when the wheal subsided, leaving a faint erythema which slowly

¹ This was not the case where a man had received a large burn, in which case his sensitivity was definitely increased.

followed the usual course of a mustard gas burn. This has been subsequently confirmed several times and tried with different samples of dichlorethylsulphide. The same samples which gave the atypical reaction on H. W. S. failed to do so on other individuals. In a number of cases with the atypical reaction a typical blister is not formed but petechial hemorrhage replaces it. This subject tested for sensitivity at this time showed a positive reaction to the vapor test in five seconds as against a previous two minutes. At the time of writing the reaction is still present. This reaction appears to be of an anaphylactic nature, a sensitization to some tissue decomposition product formed by the action of mustard gas.

TABLE 5

SPECIES	NUMBER TESTED	PERCENTAGE POSITIVE TO		
		1 per cent	0.1 per cent	0.01 per cent
Horse.....	1	100	100	100
Dog.....	91	83	35	0
Goat.....	11	55	36	0
Rat.....	10	30	20	0
Mouse.....	7	70	14	0
Rabbit.....	2	100	0	0
Guinea-pig.....	12	33	0	0
Monkey.....	9	22	0	0

The great number of tests which have been made on the same individual at different times and under the same conditions, indicate a remarkable constancy in reaction. A series of men who were tested at various times during a period of four months, revealed slight changes from time to time in some of the men. No man who originally reacted to only the 1 per cent solution ever reacted to the 0.01, and likewise, no man who originally reacted to the 0.01 ever failed to react to the 0.1 per cent. However, since most of the work was done in extremely hot weather it was difficult to control sweating.

Susceptibility of skin of animals. The paraffin oil test was used on a number of animals and indicated that differences in susceptibility exist in different species and in different individuals of the same species. Table 5 shows the results.

The horse appears to be the most sensitive and the monkey and guinea-pig the most resistant species, while the dog would seem to have a sensitivity as near man as any of the other species studied. However, the number of animals examined is too small for any far reaching conclusions, and it is to be noted that no animal exhibits a reaction at all similar to that in man. No animal has yet been found which will give a blister from the application of mustard gas.

DISCUSSION

The only factor in the general characteristics of the skin which would appear to have a distinct bearing on the question of sensitivity is the apparent thickness of the skin. The fact that negroes as a race are much less susceptible to this substance than the white race, furnishes a clue to the reason for susceptibility. Any constant differences in the skin of negroes and white men might furnish a basis for future work.

The following experiments tend to throw some light on the mechanism of absorption of mustard gas by the skin, and hence, on the question of susceptibility.

Fifteen minutes after pure dichlorethylsulphide is applied to the skin it can be almost entirely removed by long continued rubbing with kerosene. This indicates that the substance is not immediately absorbed into the deeper layers of the skin. A resistant individual whose normal minimum burning time is four to five minutes and who does not react to the 1 per cent oil solution, can be made to give a reaction to a fifteen to thirty second exposure or a 0.01 per cent oil solution by covering the area of application with a glass cup immediately after, and leaving it covered for six to eight hours.

It was found that when two vapor tests were made under identical conditions on the arm of a sensitive individual (five minutes' exposure) and immediately after the exposure the arms of two individuals were impressed on each of the exposed areas for five minutes, the burns were modified in intensity. If the recipients are respectively more and less resistant than the

donor, that burn on the donor's arm given to the more resistant man will be the mildest. This is very striking. If a sensitive individual impresses his arm alternately against burns of the same concentration and exposure on a resistant and sensitive man, the recipient receives a more severe burn from the sensitive than from the resistant man. This would indicate that the skin of a resistant individual displays a greater affinity or capacity for dichlorethylsulphide than that of a sensitive man. A tentative explanation of this phenomena can be made as follows. A three phase system is involved—the air over the skin surface constitutes the outer phase; some fatty or keratinous elements of the skin, the central phase; and a cellular portion of the skin the inner phase. The central phase is rich in lipoids and poor in water, while the inner phase is rich in water and poor in lipoids. After exposure to the vapors of dichlorethylsulphide the central phase is the absorbing agent and tends to establish equilibrium with the other two phases. On account of the lipoid nature of the central phase no damage is produced here because the compound is not hydrolyzed. On its passage from the central to the inner phase hydrolysis takes place within the cell and damage results when a sufficient concentration of hydrochloric acid is attained. The outer phase is constantly being freed from vapor by diffusion and convection currents, so more and more can evaporate from the central phase. The susceptibility of an individual depends on the relative power of the central phase to hold the poison in an inactive form (not hydrolyzed) and prevent its entry into the inner phase at a sufficient velocity to result in the formation of a toxic concentration. We do not attempt to localize the central or inner phases with any definite structure of the skin. As mustard is known to penetrate the sebaceous ducts the fat here might form one phase and the epithelial lining another.

SUMMARY

1. Methods have been developed and described for determining the susceptibility of the skin to dichlorethylsulphide.

2. Tremendous differences in sensitivity are found. One man may be about 600 times as sensitive as another.

3. A large group of white men tested showed about two to three per cent of hypersensitive men and 20 to 40 per cent of resistant men.

4. Negroes as a race are much more resistant than white men.

5. Sweating and moisture increase the sensitivity of the skin.

6. Men may become more sensitive from continued exposure to the gas, and especially when severely burned.

7. An atypical reaction is described in a man who became more sensitive from continued working with the substance; this appears to be of anaphylactic nature.

8. The skins of ordinary laboratory animals have been tested, and the monkey and guinea-pig appear the most resistant, while the horse appears to be most sensitive.

9. A tentative partial explanation for differences in susceptibility has been offered.

We desire to thank Mr. J. W. Williams, Jr., for assistance in performing a number of the experiments; also Col. W. J. Lyster and Col. W. H. Walker for making these tests possible, and Lieut.-Col. S. L. Chappelle for coöperation in conducting the tests at Edgewood Arsenal.

REFERENCES

- (1) MEYER, V.: *Über Thiodiglycol Verbindungen*. *Berichte*, 1886, xix, 3259.
- (2) For description of the course and pathology of skin lesions see Warthin, A. S. and Weller, C. V. The pathology of skin lesions produced by mustard gas. *Jour. Lab. Clin. Med.* 1918, iii, 447.
- (3) MANDEL, M., AND GIBSON, W. S.: Clinical manifestation and treatment of gas poisoning. *Jour. Am. Med. Assn.* 1917, lxix, 1970.
- DERBY, G. S.: Effects of "mustard gas" on the eyes. *Am. Jour. Med. Sciences*, 1918, clvi, 733.

DICHLORETHYLSULPHID ("MUSTARD GAS")¹

I. THE INFLUENCE OF SOLVENTS, ADSORBENTS AND CHEMICAL ANTIDOTES ON THE SEVERITY OF THE HUMAN SKIN LESIONS

TORALD SOLLMANN

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Normal course of the lesions. Dichlorethylsulphid, as is now generally known, is a very powerful and peculiar irritant. It produces successively simple erythema, cutaneous edema, extensive vesication with coagulated contents, ulceration and superficial sloughing. The effects, although severe, do not extend beyond the skin or mucous membranes.

Very small doses are effective. The substance is very rapidly absorbed and fixed in the skin. Solvents prevent the effects only if they are applied almost immediately after contact; they are practically useless after ten or fifteen minutes.

On the other hand, the effects develop quite slowly. In the human skin, they become perceptible only after an hour to two days, according to the dosage. Histologic changes, however, start much earlier, probably soon after the entrance of the poison.

With severe burns, the destructive effects grow worse for several days. In any case, the healing is a very slow process, very much slower than with ordinary burns. Even when healed, the burned skin remains abnormal at least for months.

Another striking peculiarity is the entire absence of pain or sensitiveness during the first two days. At this time even ex-

¹ These investigations were undertaken in collaboration with the Medical Advisory Board of the Chemical Warfare Service.

tensive blisters cause absolutely no discomfort. This is not due to a true anesthesia, for the sensitiveness to touch is unimpaired. Apparently, the nerves are in some way protected against the poison. A little later (on the third to fifth day), when the necrotic changes develop and the epidermis is more or less destroyed, the lesions become exquisitely sensitive to mechanical and chemical irritation. In milder cases, the edema of the skin leads to intolerable itching.

The hypersensitivity increases until the lesion has reached its acme—between five to thirteen days, according to the severity. During this time, the wound tends to become covered by a sloughing pseudo-membrane. This condition persists for about a week.

The turn toward improvement generally sets in rather abruptly between the seventh and nineteenth day, so that within two or three days the wound presents a healthy looking granulating surface, and the hypersensitiveness is practically gone. This spontaneous change must be taken into consideration in judging the success of methods of treatment.

From here on, improvement is steady, but slow and tedious, and likely to be somewhat complicated by furunculosis.

Table 1 gives the average time relations of the experimental burns. They also hold good for clinical burns.

This sketch of the course of the main clinical phenomena of dichlorethylsulphid lesions has been inserted, since it serves as a "normal" for judging the modifications produced by experimental measures. Further details of the skin phenomena are contained in the excellent descriptions of Warthin and Weller (1).

Methods of experimentation. The investigation had the purpose of studying the conditions that might affect the penetration and the toxicity of the dichlorethylsulphid.

Experiments on animals was abandoned after a few trials, since their skin does not react in the same manner as human skin, and the effects that do occur are not easily graded.

The urgency of the problem at the time appeared to justify direct experimentation on human subjects. The following students volunteered for what were often quite painful experiments:

TABLE I
Mean course of experimental lesions

	SEVERITY OF BURN	NUMBER OF EXPERIMENTS IN GROUP	RUBEFAC-TION			SWELLING			ITCHING		VESICATION			PAINFUL ULCERATION			
			First perceived	Full size	Practically gone	Present	Subsiding	Practically gone	Present		Starts	Maximum	Ruptured	Pain starts	Maximum	Painless, healing	Healing, practically stationary
Rubefaction without notable swelling (generally vapor burns).	Light*	6	1 d.	2 d.													
	Severe	5	3 h.	4 h.	3 d.												
Erythema and swelling	Light	6	12 h.	18 h.		1 d.		8 d.									10 d.
	Severe	9	1½ h.	4 h.	2 d.	1 d.	2 d.	1 w.	12 h.	13 d.							2½ w.
Vesication	Light	9	1½ h.	1½ h.		1 d.			1 d.		2 d.	3 d.	5 d.	3 d.	5 d.	7 d.	4½ w.
	Severe	7	1½ h.	1½ h.		1 d.					1 d.	1½ d.	2½ d.	3 d.	10 d.	17 d.	5½ w.
Ulceration with superficial gangrene		7	1½ h.	1½ h.		1 d.			1 d.		1 d.	2 d.	3 d.	3 d.	10 d.	15 d.	5 w.
		6	1½ h.	1½ h.					1½ h.		18 h.				13 d.	19 d.	7 w.

* Four hours, livid, white wheels.

Eighteen hours, they are surrounded by vesicles.

Fourteen to nineteen days, the burned skin sloughs off in mass.

W. P. Bowser, W. D. Cassel, S. J. A. Foerstner, S. H. Lesinger, H. H. Loucks, N. C. Wetzel. Other experiments were made on myself.

Application. The poison was applied in the form of liquid and vapor. The vapor tests were preferred whenever possible, since the results are very uniform and milder.

Vapor test. In a 1 dram homeopathic vial (about 5 cm. long and 8 mm. internal diameter) is placed a very small piece of cotton wool. On this is dropped 0.01 cc. of the poison, then another small piece of cotton, carefully wiping the mouth of the vial. This is packed down with a glass rod, and the vial left corked for an hour to one day.

In applying, the open vial is held firmly against the skin by the thumb and forefinger for five minutes. The very uniform results on blank tests, and marked difference in the presence of efficient protectives show that a more complicated technic is not necessary.

The normal reaction is an erythematous papule, that does not go on to vesication.

Alcohol Solution. 0.1 cc. of dichlorethylsulphid is diluted with 20 cc. of alcôhol (the solution must be used within an hour, as it hydrolyses fairly rapidly). Of this dilution 0.005 cc. are blown from a pipette onto the skin and spread with the point of the pipette over an area of about $\frac{1}{2}$ inch diameter. Air is then blown through the pipette over the area, thus evaporating the alcohol, and leaving a thin film of the poison.

The normal reaction of this dosage is a distinct vesicle which heals slowly. This is generally a disadvantage of the method since it limits the number of experiments that can be made on a subject. It is also difficult to spread the liquid evenly over a painted or powdered skin area.

At first stronger doses were used (0.01 cc. of 1 per cent, but these sometimes gave rise to excessive reactions).

Filterpaper test. In this, 0.1 cc. of dichlorethylsulphid is spread on filter paper so as to make an even stain, about 1 by 5 cm. This is then cut into 10 squares of 5 mm. each, so that each square represents about 0.001 cc. of dichl. These are laid on the skin and kept in place by a bandage, for one or two hours.

The reaction is altogether too severe, and I have tried this method only in connection with fairly efficient protectives.

The lesions were inspected at suitable intervals and frequently photographed. I shall dispense with the presentation of the detailed notes, and shall give only a few photographs.

Effects of Solvents. The first line of experimentation concerned the effects of water and oil. Dichlorethylsulphid is readily solu-

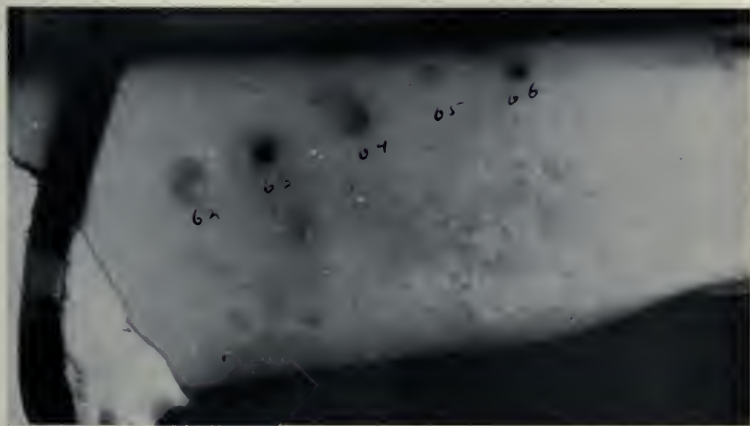


FIG. 1. DETRIMENTAL EFFECTS OF WATER (VAPOR TESTS)

Dichlorethylsulphid vapor was applied to the skin: 62, bare skin; 63, skin coated with water; 64, skin coated with sodium bicarbonate water paste; 65, skin coated with soap; 66, skin coated with kaolin water paste. The photograph was taken seven days after the application. Note that the lesion is greatest on the moistened skin, (63) as compared with the bare skin (62). Kaolin restrains this somewhat (66). Sodium bicarbonate paste (64) is useless. Soap paste (65) is fairly efficient.

ble in oil, and very little soluble in water. It therefore seemed probable that these solvents would affect its penetration and the irritation in the same way as they do phenol (2) or true mustard oil (3). For these, it was found that the irritant distributes itself between the solvent and the skin, according to its partition coefficient; the greater its affinity for the solvent, the slower will be its penetration into the tissues, and the smaller the irritation, and vice versa.

The results and experiments showed that the same principles apply to dichlorethylsulphid. Water increases the irritation (fig. 1) and oils render it less irritant (fig. 2).

Unfortunately, however, the absorption is still very rapid, even in the presence of oils, and the ultimate injury is not very much reduced. The importance of the delayed absorption is further minimized by the fact that the irritation is proportional

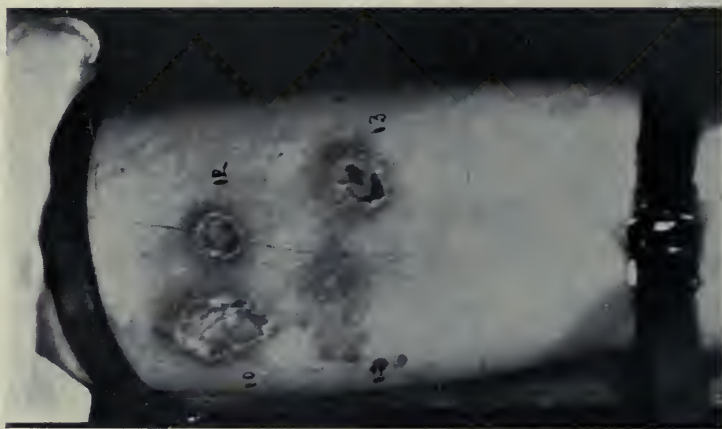


FIG. 2. PROTECTIVE ACTION OF PETROLATUM WHEN DICHCLORETHYLSULPHID IS APPLIED AS "SPASH," AND WHEN APPLIED THROUGH FABRIC

In nos. 10 and 11 the alcoholic dichlorethylsulphid was applied directly to the skin; no. 11 was previously vaselined. The protective value of the petrolatum in no. 11 is apparent. In nos. 12 and 13, the alcoholic dichlorethylsulphid was dropped on a small square of cloth and this was applied to the skin; no. 13 was first oiled with petrolatum. Note that the two lesions are practically alike. The photograph was taken nineteen days after the application.

to the absolute quantity, rather than to the concentration of the poison. This difference from most other irritants is explainable on the assumption that the toxic effects are not due to the dichlorethylsulphid itself, but to its intracellular decomposition, resulting in the liberation of hydrochloric acid within the cells.² The degree of this intracellular acidosis would, of course, depend

² The evidence for this explanation will doubtless be presented by those with whom it originated.

upon the absolute quantity of the dichlorethylsulphid that had penetrated into the cell. Since the poison probably does not leave the cells after its absorption, the *rate* of absorption would have little effect on the absolute quantity absorbed.

Notwithstanding these restrictions, however, the influence of the solvents is quite distinct, within certain limits. The protection by oils is especially efficient in prolonging the time during which removal-treatment remains effective (fig. 3).

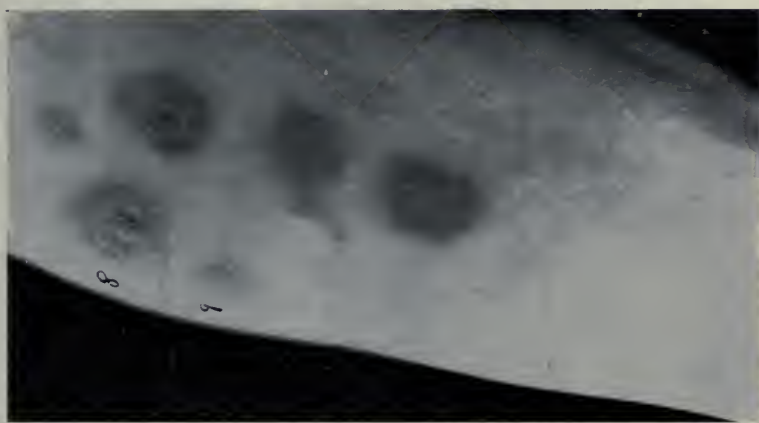


FIG. 3. VALUE OF PROTECTIVE OILING

In no. 8, the dichlorethylsulphid was applied to the bare skin, in no. 9 to oiled skin. Both were washed with oil, after fifteen minutes. Observe the much greater effect on the unprotected skin (8). The photograph was taken twenty-four days after the application.

The efficiency also increases with the thickness of the oily layer on the skin; and this depends largely on the stiffness of the oil or ointment. The efficiency is therefor increased by the addition of powders or "fillers."

This effect appears to be purely mechanical, for no material differences exist between the various substances that were tried.

There are, however, differences between the oils themselves, that are not explainable on a physical and mechanical basis, and must therefor presumably be chemical. Linseed oil, raw as well as boiled, and codliver oil furnish considerably more protection

than do other oils (fig. 4). This is perhaps due to unsaturated fatty acids. The efficiency however, is also limited (fig. 5).

Under certain conditions, the fats apparently may increase the toxicity; namely, when they facilitate the contact with the poison. This occurs, for instance, when cloth saturated with the oil is laid loosely on the skin (fig. 2).

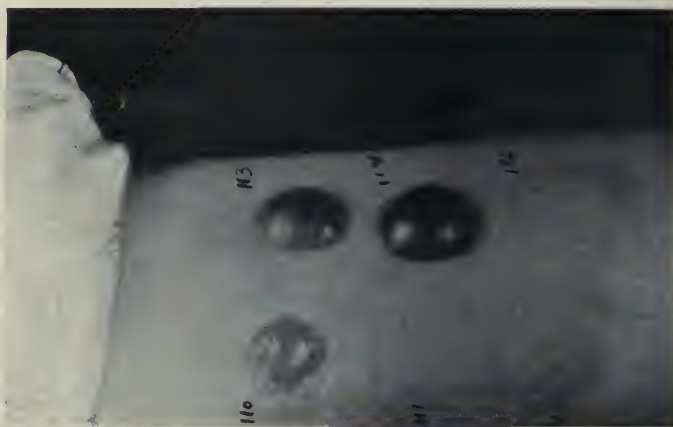


FIG. 4. COMPARISON OF OILS

Observe the degree of effect. Each area received 0.01 cc. of 3 per cent solution of dichlorethylsulphid in the oil, spread over a surface of about one-half inch diameter. The photograph was taken two days after application. The solvent oils were as follows: 110, liquid petrolatum; 111, raw linseed oil; 112, boiled linseed oil; 113, olive oil; 114, castor oil; 115, cod liver oil.

The following gives a more detailed outline of the experiments and results:

1. *Water.* The effects of dichlorethylsulphid are more severe in the presence of moisture on the skin. This is shown very convincingly by the vapor test, in experiment 63 (fig. 1).

This action of water is reflected in the attempt to use protective substances as watery solutions or pastes. These are uniformly less effective than the dry substances, and in nearly all cases they are also less effective than the oily solutions or pastes.

Because of their water content, the following rendered the skin hypersusceptible, so that the lesions were more severe, than on the

bare skin: 50 per cent glycerin (experiment 15); sodium bicarbonate-water paste (experiment 64); kaolin-water paste (experiment 66); fuller's earth-water paste (experiment 68) (see figure 1).

The deleterious effects of moistening the skin do not contradict the beneficial effects of washing and scrubbing the skin, after exposure, with soapy solution.

On the other hand, it is evident that the skin should be protected, against moisture during exposure, by keeping it covered with absorbent dusting powders; or better, by keeping it oiled, if that is practical.



FIG. 5. PROTECTIVE VALUE OF DICHLORAMINE T

Pieces of filter paper, about 5 mm. square, and containing about 0.001 cc. of 95 per cent dichlorethylsulphid were applied to the center of a square inch of skin, covered with the protectives. The coatings were as follows: 117, Raw linseed oil; 118, linseed oil and kaolin, 1:3; 119, same, with 3 per cent of soft soap; 120, petrolatum kaolin 1:1; 121, Dichloramin T, 10 per cent in chlorcosane; 122, solid paraffin. The photographs were made one day after the application. Note that the reaction is less in 121 than in the others; 26 and 30 are recrudescences of lesions twenty-five days old.

2. Simple oils and fats. All oils restrain the effects of dichlorethylsulphid. They differ quantitatively, however.

The relative protective efficiency can be seen when the vapor or alcoholic solutions are applied to the oiled skin; but they are especially striking if equal doses of a 3 per cent solution of dichlorethylsulphid in various oils is applied to the skin. The protective efficiency in the different series is in the following order, the most effective protection being at the top, the least effective at the bottom.

Vapor tests

(Sollmann)	(Loucks)
Boiled linseed oil (53)	Castor oil (90)
Liquid Petrolatum (47)	

Liquid dichlorethylsulphid applied to oiled skin

(Small dose)	(Large doses)
Petrolatum (11)	Raw linseed oil (117)
Liquid petrolatum (2, 6, 7)	Solid paraffin (122)
Olive oil (3)	
Lanolin (4)	

Dichlorethylsulphid dissolved in oils (3 per cent)

Raw and boiled linseed oil (111
and 112)

Cod liver oil (115)
Liquid petrolatum (110)
Olive oil (113)
Castor oil (114)
(See figure 4)

From these data the general order of efficiency is

Linseed oil, raw or boiled
Cod liver oil
Solid paraffin
Petrolatum
Liquid petrolatum
Olive oil
Castor oil
Lanolin

Oiling of the skin is decidedly protective against slight exposure (experiments 47, 53, 90) to the vapor, and fairly effective against small doses of the liquid (2, 3, 4, 11). Its usefulness, however, is not unlimited. Even the most effective oils do not prevent blisters if the strong dichlorethylsulphid is left in prolonged contact (117 to 122) (figure 5).

3. "*Filled ointments*. Many of the substances that were tried as chemical antidotes really act merely as fillers. The tabulations are again arranged in the order of efficiency, those giving the most complete protection are at the top; the least protection at the bottom. Some of the plain oil are given in brackets for comparison.

Vapor test, Sollmann

Zinc oleate (50)

Linseed oil (53)

Liquid petrolatum stiffened with charcoal (50) Kaolin (49) of Fuller's Earth (48)

Vapor test, Cassel

Zinc oleate (97) and solid petrolatum pastes made with

Collargol (94)

Zinc stearate (95)

Zinc oxid (96)

Manganese dioxid (98)

Silver abietate (resinate) (101)

Boric acid (99)

Solid petrolatum (100)

Liquid alcoholic dichlorethylsulphid

Zinc in liquid petrolatum (21)

Hexamethylamin in liquid petrolatum (20) (petrolatum, 11)

When the dichlorethylsulphid is concentrated, even the filled ointments have only slight values, this is shown by the series 116 to 122, which also includes solid paraffin (fig. 5). In such cases only the chlorin preparations are promising.

4. *Protective varnishes.* These may be supposed to act like the fats. They would remain longer on the skin, but they can only be applied in a very thin coat and this is likely to furnish only an incomplete protection. A thicker coating might soon become harmful.

The actual tests by the vapor method although not complete were not encouraging.

Aluminum paint (83) actually increased the irritation; asphalt-ether varnish (80) was no better than bare skin. Collodion (85) furnished a very slight protection.

The following protected, but it was not determined whether the protection was greater than that of simple oiling: Rosin-ether varnish also with zinc stearate and with fuller's earth, shellac varnish.

Adsorbent powders. These were found highly effective, just as they are in the masks. A layer of 1 mm. thick protects completely against the vapor test (fig. 6) and quite effectively against the alcoholic solution. The results were as follows:

Vapor tests on dry powders. Sollmann (bare skin-erythematous papule).

No lesion followed on coconut charcoal (29).

Slight and inconstant erythema (less than with linseed oil 53): Kaolin (27, 73), fuller's earth (28, 74).

Slight papular erythema: Zinc stearate (30) (about like liquid petrolatum 47).

Vapor tests on dry powders. Loucks (bare skin-erythematous papule).



FIG. 6. EFFICIENCY OF DRY POWDERS

Dichlorethylsulphid vapor was applied. Note the normal lesion in the bare skin (26); the slight protection afforded by zinc stearate (30); and the complete protection from kaolin, fuller's earth and charcoal (indicated by the absence of lesions in a straight line drawn from 26 to 30). The photograph was taken three days after the application.

No lesions followed on: Manganese dioxid (103), talcum (108), zinc oxid (105).

Slight erythema: Silver abietate (107), reduced iron (104), litharge (106).

Moderate erythema: Zinc stearate (107).

Liquid test with alcoholic dichlorethylsulphid. Fuller's earth protected most, talcum intermediate protection, calc. carbonate, least protection.

These results show that the general efficiency of the powders is as follows, the most effective being at the top, the least effective at the bottom of the list:

Coconut charcoal
Fuller's earth, kaolin
Talcum, manganese dioxid, zinc oxid
Silver abietate, reduced iron, litharge
Zinc stearate
Calcium carbonate

Practically the usefulness of the adsorbent powders is limited by the difficulty of keeping them on the skin in sufficient thickness.

The above tabulation of relative efficiency indicates plainly that the following act only mechanically, and not chemically, and that this efficiency is actually lower than that of the cheaper charcoal, kaolin, or Fuller's earth, namely: Metallic soaps (zinc stearate); metallic resinate (silver abietate); metallic oxids (zinc oxid and litharge); and free metals (reduced iron).

Adsorbent water pastes. The attempt was made to secure a better adhesion of the adsorbents to the skin, by using them in the form of pastes. These pastes are not nearly as efficient as the dry powders. This is explainable partly by the deleterious effects of water itself, and partly by the watery film, preventing ready access of the nearly insoluble dichlorethylsulphid to the adsorbent.

The following experiments were made, the results again being presented in order, the most efficient above, the least effective below.

Vapor tests. Sollmann: (Bare skin gives erythematous papule).

Inconstant slight erythema: Coco-charcoal paste (67) (about like dry Kaolin).

Popular erythema, rather less than on bare skin: Powdered zinc paste (71).

About as on bare skin: Fuller's earth paste (68).

Slightly more severe than on bare skin: Kaolin paste (66).

Complete vesication: Sodium bicarbonate paste (64).

(Water alone produces more severe blister and scabbing (63).

Vapor tests, Cassel. Collargol, 10 per cent (93) did not furnish protection, giving the same results as on the bare skin.

Liquid alcoholic dichlorethylsulphid. Alkresta in 50 per cent glycerin (16) furnishes a fair protection, whilst 50 per cent glycerin alone increases vesication (15).

Plain soap. "Soft soap" furnishes considerable protection, chiefly it is presumed by acting as a solvent. It acted as well as linseed oil or zinc oleate in the vapor test (65). It may also be incorporated with the ointments, for instance 5 per cent with petrolatum (91); and 3 per cent in the linseed kaolin mixture (119). The addition of the soap modifies ointments so that they can be applied more smoothly and also facilitates their removal by washing. These advantages, however, are not very great and it is conceivable that the alkalinity of the soaps might injure some skins.

Metallic soaps. Certain reports by other workers indicated that metallic soaps and resinates would be especially effective. This was not confirmed. Those tried by me had only a limited success, and this was attributable mainly to their mechanical action as adsorbents and fillers.

Lead plaster (54): protected well in the vapor test, but this was explained by the dense consistence of the film.

Zinc oleate (the old U. S. P. preparation): protected partially in the vapor test, being about equal to linseed oil (53) and very little better than kaolin-petrolatum ointment (50). On another subject (97) it protected completely, but so did ointment of zinc oxid or boric acid.

Zinc stearate in the dry form (30,107): protects very little, even against vapor, much less than dry kaolin (28), talcum (108), or zinc oxid.

Zinc stearate petrolatum ointment: Furnished protection against vapor (95) but so did similar ointments of zinc oxid (96) and boric acid (99).

Silver abietate (resinate), dry: This furnished only a relatively slight protection against vapor (107) being inferior to talcum (108).

Silver abietate-petrolatum ointment: This protects against the vapor (101); but so did a similar boric acid ointment (99).

Powdered and colloidal metals and metallic oxids. These were tried in the hope that they might act as catalysts, accelerating

the hydrolysis of the dichlorethylsulphid. The results show that this does not occur under the conditions of their use on the skin. They furnish some protection, but no more than any indifferent powders. The following were tried:

In dry powder form. Powdered zinc (79): Protection against vapor much less than dry kaolin (73).

Manganese dioxid (103) and zinc oxid (105): Protect against vapor, but so did talcum (108).

Iron (reduced), and litharge (104): Protect against vapor less efficiently than talcum (108).

As water pastes. Zinc dust paste (71): Protects very little better than fuller's earth paste (68), both being about the same as on the bare skin (62).

Collargol, 10 per cent (93): Does not protect.

As petrolatum ointments. Zinc dust ointment: Protects somewhat better than petrolatum; but probably not better than any indifferent powder, such as Hexamethylanamin (20).

Manganese dioxid ointment (98), zinc oxid ointment (96) and collargol ointment (94): Protect against vapor, but so did boric ointment (99).

Chlorin preparations. The caustic action of dichlorethylsulphid is destroyed by further chlorination. Chlorinated lime and the chloramins are effective under working conditions. Their efficiency however is limited; and their practical application is further confined by their irritant action on the skin, the instability of some of the preparations, and the cost of the chloramins.

Their efficiency is illustrated by figure 5 and by the following experiments:

In the vapor tests. Complete protection was secured by:

Dichloramine T, dry (76), or in water-paste (69) or as 10 per cent dusting powder (78) and 10 per cent in chlorcosane.

Calx chlorinata, in the same forms (75, 70, 77). The efficiency of Dichloramine T and calx chlorinata was equal to that of dry charcoal, and superior to all others.

Chloramin T paste, Squibb (52) gave almost complete protection.

Against Concentrated Liquid G34, Dichloramin T, 10 per cent in chlorcosane gave only partial protection (121), but was somewhat more efficient than linseed kaolin pastes (118, 119). This is shown in the figure 5.

REFERENCES

- (1) WARTHIN AND WELLER: 1918, J. Lab. Clin. Med. iii, no. 8.
- (2) SOLLMANN: (Phenol), 1916, J. Amer. Med. Assoc., March 17.
- (3) SOLLMANN: (Mustard Oil), J. Exp. Pharmacol. Therap. xi, 229.

DICHLORETHYLSULPHID ("MUSTARD GAS")

II. THE QUESTION OF INDUCED HYPERSUSCEPTIBILITY OF THE SKIN

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"Sores" and catarrhs. Workers on "mustard gas" very generally have the conviction that they have become hypersusceptible. This appears to be based on frequent occurrence of "sores," which are however furuncles and not true mustard lesions; and of chronic conjunctival or laryngeal catarrh, which may well be cumulative results of continued exposure to low concentrations, rather than evidence of hypersusceptibility.

Healed areas. Another form of hypersusceptibility is seen in the "healed" areas of previous burns. "Mustard" burns heal much more slowly than ordinary wounds. The skin over the healed burns has a peculiar appearance as long as it has been under observation; and it is highly susceptible to any kind of irritation. Slight contusions or other mechanical injuries readily produce blisters; and so does chemical irritation; and dichlorethylsulphid among others. Figure 5 of the first paper illustrates how some old lesions, that had been "completely healed" for weeks, were blistered by exposure to a vapor that produced only erythematous swelling of the surrounding skin.

Sensitivity test. None of the above observations answer the question whether untouched areas of the skin become hypersusceptible. This would require direct tests such as the sensitivity test introduced by E. K. Marshall, which consists in ascertaining the minimal effective concentration of dichlorethylsulphid when dissolved in liquid petrolatum. Subjects that respond by definite erythema to a 0.01 per cent solution within

forty-eight hours are considered as "hypersusceptible;" whilst no reaction after 1 per cent is considered "resistant."

The question of hypersusceptibility could be answered by applying this test before and after a man has worked with the poison. Unfortunately, this phase of the subject was not taken up until too late, so that I do not have both sets of observations. However, it is interesting to record that the patient with the most severe burns who came under my observation was not hypersusceptible after the burn. This shows that hypersusceptibility, if it exists at all, is not an invariable phenomena.

A private (Smith) who had worked about three months on dichlorethylsulphid, had several minor burns, and then a very severe and extensive burn on August 13. The sensitivity test (0.01 per cent) was applied six weeks later, when nearly all the lesions were healed, to unburned skin, and to a healed blister. Neither showed even a reddening.

Another test was made on a subject during the acute stages of a dichlorethylsulphid burn (about the third day). Both hands were covered with large blisters. Application of 0.01 per cent to the arm gave no response. The susceptibility need therefor not be increased even during the active stages of the burn.

Dermatographia. The skin of the untouched areas of all the subjects of "mustard" burns gave a marked dermatographia. However, since this could not be measured quantitatively, it is not certain that the vascular irritability was really increased.

Luetin test. This was originally proposed as a specific test for syphilis, and has been shown to be a striking index to a certain kind of acquired skin hypersusceptibility. It is induced especially by iodine compounds and related products. It appeared interesting to determine whether this hypersusceptibility would also be induced by dichlorethylsulphid.

Following this suggestion, Dr. H. N. Cole on August 15 applied the Luetin test to six patients at the Cleveland Marine Hospital. None gave a positive response.

All the subjects had had extensive and severe burns, from two days to two and one-half months before the test. Two of the

subjects had worked on mustard for three months, another for two months.

Summary. "Healed" dichlorethylsulphid burns are over-susceptible to injury, mechanical or chemical, and therefor incidentally to dichlorethylsulphid.

Prolonged work with dichlorethylsulphid and severe and extensive burns of the skin do not render the uninjured areas of the skin hypersensitive, either to dichlorethylsulphid itself, or to luetin.

THE EFFECT OF COCAINE HYDROCHLORIDE ON THE CO₂ PRODUCTION OF THE MIXED NERVE FIBER

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I. INTRODUCTION

There are many different theories advocated concerning the mechanism by which a tissue is rendered temporarily non-irritable. Recently considerable controversy has arisen over the question whether or not narcosis might be the result of asphyxiation. Various results obtained by many investigators on the relation between oxidation and narcosis may be summarized into three different classes:

1. Narcosis is due to an interference with oxidation of the tissue.
2. Narcosis can be produced without practically interfering with the normal intake of oxygen.
3. Though the rate of oxidation may be interrupted during narcosis, yet there must be a still more fundamental causation, which is the factor primarily responsible for the phenomena, but to which changes in oxidation are merely incidental.

Verworn (12) is well known as one of the strongest exponents in advocating the idea that narcosis is primarily due to asphyxiation. Mansfeld (5) and many others also came to a similar conclusion.

Warburg (13), on the other hand, observed that phenylurethane inhibited segmentation of the eggs of the sea urchin without any remarkable interference with the rate of oxidation in the eggs. Loeb and Wasteneys (3, 4) also contend that lowering the rate of oxidation in the eggs, when treated by many narcotics,

cannot be accounted as the cause of narcosis. Their later works on *Fundulus* and medusae led to the hypothesis that an incidental diminution of the rate of oxidation, caused by specific narcotics, was either a result of inactivity of muscles (or other organs) of the medusae which was caused by narcosis, or a secondary action, having no important effect in bringing about narcosis.

Winterstein (14, 15, 16, 17) first supported the idea that the phenomena are produced by nothing but diminution of oxidation, but he now concludes from a series of later experiments that the decrease in the rate of the oxidation under this condition must be due to other causes, which should not be used as an explanation of narcosis.

Traube (7, 8), on the other hand, advanced the idea that all narcotics have one common physico-chemical characteristic, that is, they are "Stoffe von geringen Haftdrucke in Wasser," possessing a power to lower the surface tension of water and internal pressure in the cell. Such lowering of surface tension must produce marked changes in the physical state of the dissolved substances, e.g., colloidal state. These changes must immediately affect the chemical processes, the foremost of which must be tissue oxidation. He attempts, thus, to explain the finding of Verworn's school as results of secondary phenomena which are primarily brought about by changes of the purely physical condition of the cell by narcotics.

Tashiro and Adams (11), testing the effects of ethyl urethane and choral hydrate on CO_2 output of the claw nerve of the Spider Crab by means of the Biometer, record that CO_2 production is greatly diminished when the nerve is narcotised by these substances in concentrations which produce reversible loss of its excitability, while, with a weak concentration of these narcotics, CO_2 output was increased. While, thus, they do not attempt to explain how narcosis is brought about, they prove that the idea that the rate of respiratory activity remains undisturbed during narcosis is entirely incorrect, as far as concerns the nerves they studied. They suggest that

A consideration of these facts, together with various other findings of Mathews, Loevenhart, Verworn and others, strongly suggests that primarily the effect of a narcotic on protoplasm may be to produce a change in its chemical instability, from which a change in the metabolic rate and loss of irritability might be brought about by replacing oxygen by a weaker oxidizing substance, or by rendering the protoplasm less oxidizable through a union of protoplasm and narcotics, as suggested by Professor Mathews.

Later, Tashiro (10) has shown that along comparatively pure nerve fibers, there is a distinct metabolic gradient which is directly associated with the natural functional direction; i.e., in all the fibers he examined, he found that in afferent fibers, there is always more CO_2 produced at the distal portion of the fibers than at the proximal portion for equal weight; and in efferent fibers, the reverse is the case. He further noted that not only this metabolic condition is closely connected with the functional gradient in the nerve but that there is a variety of conditions which can alter this gradient. Anesthetics, among other things, when used in proper concentrations alter or reverse this metabolic condition. Professor Tashiro¹ suggested to me, therefore, that it would be of great interest to investigate the metabolic condition of the mixed nerve when treated by so-called local anesthetics. The original plan to use as many local anesthetics as available has not been carried out on account of circumstances which compelled the author to leave this country. Hence the record to be reported here is confined solely to the action of cocaine hydrochloride on the mixed nerve.

II. EXPERIMENTAL

1. *Materials and methods*

The best specimens of cocaine hydrochloride made by Kahlbaum and by Merck were used. The alkaloid was made up in Ringer's solution to 5 per cent and a desired concentration was made by diluting this original solution by Ringer's solution.

¹ For this and other suggestions, I am greatly indebted to Prof. Shiro Tashiro.

(NaCl 49 grams, KCl 2.1 grams, CaCl_2 1.75 grams made up to 1000 cc. with a doubly distilled water.)

The sciatic nerve of frogs (*Rana pipiens*) were exclusively used, not only for the reason that their metabolic activities are well investigated, in this laboratory, but also because the ratio between afferent and efferent fibers along the different parts of the sciatic is also known. In isolating the nerve, the usual precautions such as the prevention of drying effects, and other accessory and unnecessary injurious effects, have been taken.

The excitability of the nerve under treatment with the drug was determined by means of the Du Bois-Reymond's inductorium, no other quantitative measurements being made of the strength of stimulus, than by noting distances of the secondary coil.

The sciatic nerve of the frog was isolated in the usual manner, and a muscle-nerve preparation was made. Only the nerve was immersed in the solution, care being taken that the muscle did not come in contact with the solution. The excitability was judged by the contraction. Thus our results, all the way through, refer the loss of excitability to the loss of the excitability of the efferent fibers. As to the condition of afferent fibers, I have no first hand knowledge, although it seems to be fairly well established that afferent fibers are first to be narcotized. Therefore, anesthesia, described in all cases, refers in a strict sense to the efferent fibers only.

The CO_2 production of the nerve was determined by Tashiro's biometer (10), the details of the method being described by Tashiro in the references cited. The nerve was isolated in the usual manner, weighed to a milligram, and then immersed in the solution for a certain desired length of time. At the end of immersion, adhering liquid was removed by means of filter paper, and the rate of carbon dioxide production was determined as usual.

For all experiments, a control experiment was performed with the nerve treated with Ringer's fluid only. It is plain that since a little difference in conditions such as temperature, the rate of drying, the condition of laboratory, and other unexpected dis-

turbances, often markedly affects CO_2 production of the nerve, a strict comparison with the control under the same condition is indispensable.

2. Results of the experiments

(a) CO_2 production of normal isolated sciatic nerve of frog. In order to make the comparison a rigid one, the normal nerve was subjected to a treatment exactly similar to that employed with the anesthetized nerve. It was isolated in the usual manner, quickly weighed to a milligram, and immersed in Ringer's solution. After the nerve was treated with the solution for the following lengths of time: ten minutes, thirty minutes and one hour, it was placed in the biometer, and the amount of CO_2 produced from the nerve was determined. According to the results, the longer the time that elapses after the nerve has been isolated, the less is the amount of CO_2 produced from the nerve.

The results are indicated in table 1.

TABLE 1

CO_2 production from isolated sciatic nerve of frog treated with Ringer's solution

TREATMENT	TIME IN MIN- UTES	CONDITIONS OF THE NERVE	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. NERVE IN TEN MINUTES
Ringer's solution....	10	Excitable	No change	6.4×10^{-7} g. at 18°
	30	Excitable	No change	6.3×10^{-7} g. at 19°
	60	Excitable	No change	6.0×10^{-7} g. at 19°

It should be pointed out here that the amount of the CO_2 output by the nerve after one hour's immersion in Ringer's solution is much greater than the amount reported by Tashiro.² The apparent disagreement between two figures, however, is not entirely due to experimental errors. A careful comparison of the two experimental methods will show that there are at least two differences in technique, which will make my figure much higher, mainly due to the more favorable conditions, for the respiration of the nerve.

In the first place, temperatures at which the experiments were performed were quite different. The temperature at which

² Page 117, loc. cit

Tashiro's experiments were performed was 25°C, while that of mine was 19°C. Although it is true that, other conditions being equal, a nerve would give off more CO₂ at a higher temperature, yet it is also to be expected that an isolated nerve would have a greater speed of death rate at a higher temperature than at a lower temperature. The exact quantitative determinations of this point, however, are under investigation in this laboratory and the results are to be reported later.

The last difference between the two methods is the matter of respiration period. Our analysis was conducted for ten minutes respiration in the respiratory chamber, while Tashiro, on account of a very low rate of CO₂ production, was obliged to let the nerve respire for a much longer period than ten minutes. How far this difference of the respiration period did contribute toward widening the gap between our figure and his was not investigated.

It is true, however, for an isolated nerve, that a longer respiration period always tends to make a lower average rate of CO₂ output for a unit of time due to a gradual decrease in the rate of respiration, rather than due to a fault of the method.

All these things considered, it is not surprising to see our figure to be much higher than his. It should be recalled that our interest in determining the rate of CO₂ output of the nerve treated by Ringer's solution was to have standard figures of the control nerves to which our subsequent data from anesthetized nerves could be compared, thus working under exactly identical conditions for both treated and untreated nerves.

(b) *CO₂ production from isolated sciatic nerve of frog treated with a 5 per cent cocaine hydrochloride solution.* A freshly isolated sciatic nerve of the frog was immersed in this solution for different periods of time namely, ten minutes, thirty minutes, and one hour. The nerve thus treated with this drug for ten minutes was completely anesthetized, and then on return into Ringer's solution, the excitability of the nerve is restored to a normal condition apparently with no injurious effects. When treated for thirty minutes in this concentration, the conditions of excitability of the nerve only partially or incompletely returned to normal when replaced to Ringer's solution. The

CO₂ output of the nerve treated for ten minutes was only a little diminished, while that of the nerve immersed in this solution for thirty minutes or one hour was remarkably decreased. Further, it was found that the longer the nerve was treated with this cocaine hydrochloride, the greater the diminution of CO₂ production. The weight of the nerve was remarkably altered by immersion in this concentration. The change of weight was independent of the length of time during which the nerve was treated under this condition.

These results are indicated in the table 2.

TABLE 2

Effects of a 5 per cent cocaine hydrochloride solution on the isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO ₂ PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
5 per cent cocaine hydroch. solution	10	Completely anesthe- tized	Normal	Loss of 6 to 9 per cent	6.2×10^{-7} g. at 18.0°
	30	Completely anesthe- tized	Partially or incom- pletely recov- ered.	Loss of 6 to 9 per cent	5.0×10^{-7} g. at 18.5°
	60	Completely anesthe- tized	Partially or incom- pletely recov- ered.	Loss of 6 to 9 per cent	4.3×10^{-7} g. at 19.0°

(c) CO₂ production from isolated sciatic nerve of frog treated with a 2.5 per cent cocaine hydrochloride solution. As shown in the table 3, the isolated sciatic nerve of the frog treated with this concentration for ten minutes was partially anesthetized, while the nerve immersed in this solution for thirty minutes or for one hour lost completely its excitability, but its excitability was easily restorable. The amount of CO₂ produced from such a nerve treated with a 2.5 per cent cocaine hydrochloride solution for ten minutes was a little more than that from the normal nerve, and the CO₂ output of the nerve similarly treated for

thirty minutes or one hour was diminished below that of the normal nerve. In this case also, the longer the nerve was treated, the greater was the decrease in CO_2 production. Thus, the nerve treated with this concentration for thirty minutes and completely anesthetized produced at 19.0° 6.2×10^{-7} grams of CO_2 per centigram of the nerve for ten minutes respiration, while the nerve similarly treated for one hour and perfectly anesthetized produced at the same temperature 5.4×10^{-7} grams per centigram of the nerve for ten minutes respiration. The difference between the amount of CO_2 produced from the nerve treated with this solution for thirty minutes and that produced from the normal nerve placed otherwise under the same conditions was very small. The nerve also lost weight by treatment with this concentration, but a little less than the nerve treated with a 5 per cent cocaine hydrochloride solution. In this case it was also independent of the length of time the nerve was immersed in the anesthetics.

The results are indicated in table 3.

TABLE 3

Effects of a 2.5 per cent cocaine hydrochloride solution on isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
2 per cent cocaine hydroch. solution	10	Partially anesthe- tized	Normal	Loss of 4 to 6.5 per cent	6.9×10^{-7} g. at 18°
	30	Completely anesthe- tized	Normal	Loss of 4 to 6.5 per cent	6.2×10^{-7} g. at 19°
	60	Completely anesthe- tized	Normal	Loss of 4 to 6.5 per cent	5.4×10^{-7} g. at 19°

(d) CO_2 production from isolated sciatic nerve of frog treated with a 1 per cent cocaine hydrochloride solution. As indicated in table 4, the nerve which was treated with 1 per cent cocaine solution for ten or thirty minutes was anesthetized only partially, and, on return into Ringer's solution, it was perfectly restored, while the nerve treated for one hour was completely anesthetized, and,

brought into Ringer's solution, recovery of its excitability was apparently perfect. The amount of CO_2 production of the nerve immersed in this anesthetic for ten or for thirty minutes was more than that of the normal nerve while the CO_2 output of the nerve treated with this solution for one hour was less than that of the normal nerve. Thus the CO_2 production of the nerve partially anesthetized was increased, while that of the nerve completely anesthetized was diminished. The amount of CO_2 production of the nerve immersed in this solution for ten minutes was more than that of the nerve treated with the same concentration for thirty minutes. It is found in this case, also, that the longer the nerve is treated with this solution, the less is the CO_2 output of the nerve. The loss of weight of the nerve thus treated was about 2.5 to 5 per cent, which did not vary much in treatments from ten minutes to one hour.

Table 4 indicates these results.

TABLE 4

Effects of a 1 per cent cocaine hydrochloride solution on isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
1 per cent cocaine hydroch. solution	10	Partially anesthe- tized	Normal	Loss of 2.5 to 5.0 per cent	7.1×10^{-7} g. at 17.0°
	30	Partially anesthe- tized	Normal	Loss of 2.5 to 5.0 per cent	6.5×10^{-7} g. at 17.5°
	60	Completely anesthe- tized	Normal	Loss of 2.5 to 5.0 per cent	5.8×10^{-7} g. at 20.0°

(e) CO_2 production from isolated sciatic nerve of frog treated with a 0.5 per cent cocaine hydrochloride solution. As shown by the physiological tests, the nerve treated with 0.5 per cent cocaine solution for ten or thirty minutes was partially anesthetized, and, brought into Ringer's solution, its excitability was restored, while the nerve similarly treated for one hour was completely anesthetized, yet the recovery of its excitability was com-

plete. The CO_2 output of the nerve treated with the same concentration of cocaine for ten or for thirty minutes was more than that of the normal nerve. But the nerve immersed in this solution for one hour gave off a little less CO_2 than the normal. Thus the CO_2 production of the nerve partially anesthetized was increased, while that of the nerve completely anesthetized was slightly decreased. Furthermore it was found that the CO_2 output of the nerve treated with this drug for ten minutes was more than that of the nerve similarly treated for thirty minutes. Loss of weight of the nerve immersed in this concentration was about 2 to 3 per cent, and bore no relation to the length of time the nerve was immersed in this solution.

These results are indicated in table 5.

TABLE 5

Effects of a 0.5 per cent cocaine hydrochloride solution on isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
0.5 per cent cocaine hydroch. solution	10	Partially anesthe- tized	Normal	Loss of 2 to 3 per cent	7.0×10^{-7} g. at 18.5°
	30	Partially anesthe- tized	Normal	Loss of 2 to 3 per cent	6.5×10^{-7} g. at 19.0°
	60	Completely anesthe- tized	Normal	Loss of 2 to 3 per cent	5.8×10^{-7} g. at 19.0°

(f) CO_2 production from isolated sciatic nerve of frog treated with a 0.25 per cent cocaine hydrochloride solution. The nerve treated with this solution for ten or for thirty minutes could not be anesthetized, while the nerve treated similarly in this solution for one hour was partially anesthetized, and, on return to Ringer's solution, it recovered its normal condition of excitability. The amount of CO_2 production of the nerve thus treated for either ten or thirty minutes or one hour was more than that of the normal nerve, and it was found that the longer the nerve

was immersed in this drug, the greater was the diminution of the CO_2 output of the nerve. Loss of weight of the nerve treated with this solution of the alkaloid was slight. This loss in weight was independent of the length of time during which the nerve was immersed in this solution.

The results are shown in table 6.

TABLE 6

Effects of a 0.25 per cent cocaine hydrochloride solution on isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
0.25 per cent cocaine hydroch. solution	10	Not anes- thetized	Good	Loss of 1 to 2.5 per cent	6.8×10^{-7} g. at 17.9°
	30	Not anes- thetized	Good	Loss of 1 to 2.5 per cent	6.5×10^{-7} g. at 16.5°
	60	Partially anesthe- tized	Normal	Loss of 1 to 2.5 per cent	6.5×10^{-7} g. at 17.0°

(g) CO_2 production from isolated sciatic nerve of frog treated with a 0.1 per cent cocaine hydrochloride solution. As shown in table 7, the effects of a 0.1 per cent cocaine solution on the isolated sciatic nerve of the frog was approximately the same as with a 0.25 per cent solution. The nerve which was immersed in this solution for ten or for thirty minutes was not anesthetized, while the nerve treated with the same solution for one hour was partially anesthetized, though less than that produced by a 0.25 per cent solution. This partial loss of excitability could very easily be restored. Sometimes however the nerve immersed even for one hour was not at all anesthetized. It is obvious from these data that more CO_2 was produced by the nerve which was treated with this concentration for either ten or thirty minutes or one hour, than by the normal nerve. It should be pointed out here that the amount of CO_2 production of the nerve thus treated with a 0.1 per cent solution for ten minutes was practi-

cally same as that of the one treated for thirty minutes. This indifference, however, is apparently insignificant, since the latter was determined at a little higher temperature than the former, the CO_2 output of the latter must be a little less than that of the former because the nerve usually undergoes somewhat higher rate of metabolism at the higher temperature. Thus we may safely expect that the longer the nerve was immersed in a 0.1 per cent solution, the greater the diminution of the CO_2 output as we found in all the other concentrations. It is interesting to note that the amount of CO_2 produced from the nerve treated with a 0.1 per cent solution for one hour was more than that of the nerve treated with a 0.25 per cent solution for the same duration at the same temperature. A slight loss of weight of the nerve was apparent by this treatment. Again the loss of weight was not related to the length of time during which the nerve was immersed.

The results are shown in table 7.

TABLE 7

Effects of a 0.1 per cent cocaine hydrochloride solution on isolated sciatic nerve of frog

TREATMENT	TIME IN MIN- UTES	EFFECTS ON EXCITABILITY	EXCITABILITY AFTER RETURN TO RINGER'S SOLUTION	CHANGE IN WEIGHT	AMOUNT OF CO_2 PRODUCED BY 10 MGM. OF NERVE IN TEN MINUTES
0.1 per cent cocaine hydroch. solution	10	Not anes- thetized	Good	Loss of 1 to 2 per cent	6.8×10^{-7} g. at 17.0°
	30	Not anes- thetized	Good	Loss of 1 to 2 per cent	6.8×10^{-7} g. at 18.5°
	60	Partially and very slightly anesthe- tized (Some- times not anesthe- tized)	Very quickly becomes normal	Loss of 1 to 2 per cent	6.5×10^{-7} g. at 17.0°

Summarizing the results, we may state that various concentrations of the cocaine hydrochloride solution produce different changes in the CO_2 production of the mixed fibers of the sciatic nerve of the frog. In general, cocaine hydrochloride produces parallel changes in the CO_2 output and excitability. The experimental data are summarized in the following figures (figs. 1 and 2).

III. DISCUSSION

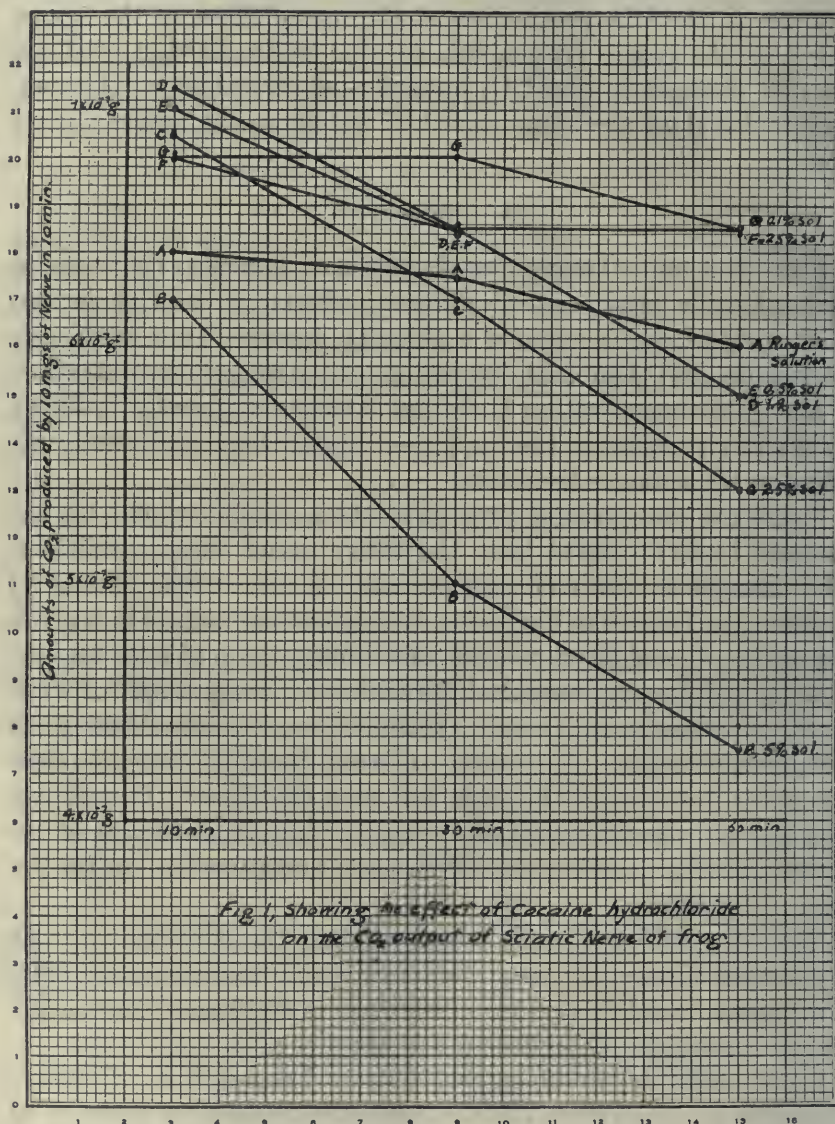
From the general summary given above, it is clear that cocaine hydrochloride produces two different effects on the CO_2 output of the sciatic nerve, namely a primary increase by a weak concentration, and then final depression by a stronger concentration. If a proper concentration is chosen, a primary increase is shown by a short treatment, then apparent restoration, and finally depression by a longer treatment.

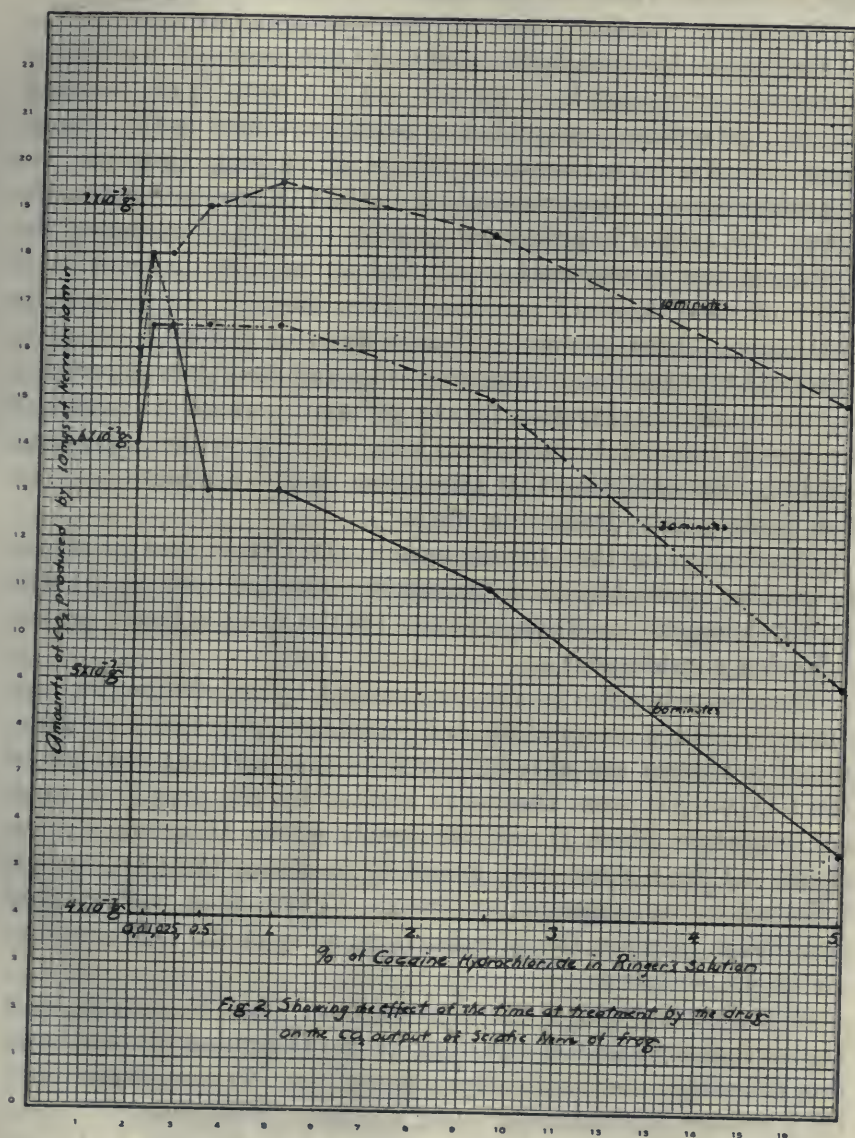
There are two possible processes which might account for the changes in CO_2 output from the nerve under these conditions. The changes in the CO_2 output may be due to the change of physiological states in the nerve, or mechanical factors independent of the physiological process, or a combination of both factors.

As is well known, cocaine hydrochloride, being a salt of weak base and strong acid, is easily hydrolyzed to a certain extent when dissolved in water. According to Gross, the salt of cocaine does not enter into the cell as such, but as a free base. He believes that the anesthetic power of the salt of local anesthetics is due to the anesthetic power of its free base, and therefore on the degree of hydrolysis.

Thus a question may be raised whether the effect on the CO_2 production altered by treatment in cocaine hydrochloride solution might be solely due to an increase of hydrogen ion concentration produced by hydrolysis of the drug.

It is probably to be expected that inasmuch as Tashiro's biometer is sensitive to such an exceedingly minute quantity of CO_2 , a slight increase of CO_2 might be easily due to the mechanical effect of hydrogen ions, setting forth CO_2 , by decomposing bicarbonates which are already present in the tissue. Such in-





creases of CO_2 would be quite independent of the normal metabolic condition, which we are measuring in terms of CO_2 .

The fact is, however, that the free hydrogen ion concentration of the cocaine hydrochloride, used in 0.1 per cent to 5 per cent solutions never reaches $P_H = 5.7$ ($H^+ = 1.9 \times 10^{-6} N$) at the maximum. According to unpublished results obtained in this laboratory from the experiments on effects of varying hydrogen ion concentrations in Ringer's solution on the CO_2 production from the nerve, the author is permitted to state that the amount of the hydrogen ion concentration we find in cocaine hydrochloride solutions used does not produce curves similar to those given in this paper. Furthermore, if it were due to a purely mechanical effect of hydrogen ions, primary increase and secondary depression by the weak concentration for the different periods of treatment are very difficult to be accounted for. These considerations warrant the conclusion that the change in CO_2 production, is not due entirely to hydrogen ion effect.

The conclusion that the changes in the CO_2 output produced by cocaine hydrochloride is intimately connected with the changes in physiological condition affected by the drug is based on the following considerations.

The characteristic phenomena caused by anesthetics in producing primary stimulation and secondary depression of functional activities are also indicated in the different changes of the CO_2 output from the nerve treated by the drug. A weak concentration which is not sufficiently strong to produce anesthesia within a short time generally increases the CO_2 output, followed by a subsequent decrease. There is a well marked parallelism between the functional capacity of the nerve and its metabolic activities.

In the second place, the depression of the CO_2 output by a higher concentration of the drug at the beginning of anesthesia is of the same order as the depression of the CO_2 output produced by a weaker concentration after a longer treatment.

Since the decrease of the CO_2 output at the beginning of anesthesia by cocaine hydrochloride is not so great as in the cases reported by Tashiro and Adams, one may raise an objection

that the depression of the metabolism is too small to account for a temporary cessation of irritability. It has been often pointed out by some authors that there are many agents and conditions which can depress the metabolism to a degree even greater than that produced by anesthetics without producing the characteristic state of anesthesia. The temperature coefficient of oxidation, for instance, is usually more than 2, but one can often lower the temperature ten degrees, thus cutting respiration more than half, without producing anesthesia. Therefore it has been contended that the depression of the oxidation cannot cause narcosis.

It is interesting to note, however, that these experiments, in which the rate of oxidation was reported to be apparently uninterrupted during anesthesia, are more striking than cases when the organs or organisms used had multiple functional activities, while the criterion used for anesthesia was based on one single functional activity, e.g., division of cells. The assumption that the rate of oxidation in such a system is homogeneous is shown to be incorrect. The normal differences of rate of oxidation along a whole tissue are necessary for an orderly performance of the functional power. Child (1) for instance, has demonstrated in many forms that the suppression of one part of oxidation, or disturbing the general order of normal metabolic gradient in the body, produced quite marked morphological pictures without killing the animal.

As cited before, Tashiro has shown that even in the nerve fibers, which, to our present knowledge, possess only one function, i.e., conduction of the nerve impulse, there is a well defined gradient of oxidation and that the effect of drugs and other agents on the fibers depends not only on the metabolic condition of the nerve as a whole, but also and to a greater degree on the different state of oxidation of the different parts of the tissue. Thus, for instance, corresponding nerves of various Crustacea behave differently toward the same concentration of an anesthetic. These differences of behavior are closely related to the different rate of the nerve impulse which in turn depends on the different rates of metabolism in nerves. Furthermore, it was

shown that the distal portion of afferent fibers which normally produce more CO_2 than the proximal portion, can be made to produce less CO_2 than the proximal portion if a proper concentration of an anesthetic is applied to the whole nerve. The normal metabolic gradient is thus entirely and easily reversed. In other words, the action of anesthetics on practically pure fibers, depends on two factors, namely general metabolic activity of the whole nerve, and the metabolic gradient of the different portions of the nerve. Dr. Tashiro suggests that anesthesia is produced in the nerve not by simply lowering the metabolism of a nerve as a whole but by primarily changing the metabolic relations of the different parts of the nerve. It was shown that the purer the fibers are, the more pronounced is the effect on oxidation which can be produced by anesthetics.

In the case of mixed nerves, when the irritability of efferent fibers alone was used as sign of anesthesia the exact state of nerve irritability of a whole nerve is very difficult to determine. Therefore, unless we can show by experiments, how much CO_2 is due to afferent and how much to efferent fibers, the exact degree of disturbance of the metabolism during anesthesia might be masked, showing only an algebraic sum of the total changes. The facts, as emphasized by Tashiro and Adams, should be recalled that the primary injury effect of high concentration of drugs when the reversibility of irritability is at the border line, is to temporarily increase instead of to decrease the CO_2 output. This is an additional factor in explaining the fact that in the nerve treated by 5 per cent cocaine hydrochloride solution which produces narcosis within a very short time, the depression of the CO_2 is not as great as we might otherwise expect.

It is a grave danger to draw generalized summary conclusions from the work done on a whole organism, in which only a single functional manifestation or a change in a simple anatomical unit is used as a sign of anesthesia. To the mind of the author, therefore, it seems to be perfectly justifiable to state that there is no evidence yet published to warrant the conclusion that changes in functional capacity can occur without corresponding changes of metabolic activity in the living system under examination.

IV. SUMMARY

1. The isolated sciatic nerve of the frog (*Rana pipiens*) when treated with cocaine hydrochloride solutions in concentrations which are not strong enough to anesthetize within a given time produces more CO_2 than that of the normal nerve, i.e., the nerve treated with Ringer's solution under exactly the same conditions.

2. If, however, there are used the concentrations which produce a state of complete but reversible anesthesia, the nerve metabolism is reduced to a varying degree depending on the depth of anesthesia.

3. The longer the isolated nerve is treated with the drug, the greater is the decrease of the CO_2 output. This is true for practically all concentrations examined.

4. In general, the statement is supported that the local anesthetics, cocaine hydrochloride, affects the metabolism of the mixed nerve in exactly the same manner as do general anesthetics, namely, primary stimulation by a weaker concentration, and subsequent diminution of the CO_2 production by a higher concentration.

5. These facts demonstrate that there is a close relationship between the rate of nerve metabolism and the state of excitability of the nerve and suggest that anesthesia in general is probably brought about by interference with the tissue metabolism.

REFERENCES

- (1) CHILD, C. M.: Senescence and rejuvenescence. Chicago, 1915.
- (2) GROS, O.: Arch. f. exp. Pathol. u. Pharmacol., lxiii, 1910.
- (3) LOEB, J., and WASTENEYS, H.: Journ. of Biol. Chem., xiv, no. 5, 1913.
- (4) LOEB, J., and WASTENEYS, H.: Biochem. Zeitschr., lvi, 1913.
- (5) LOEWE, S.: Biochem. Zeitschr., lvii, 1913.
- (6) MANSFELD, G.: Arch. f. d. ges. Physiol., cxxix, 1909.
- (7) MEYER, H., and GOTTLIEB, R.: Die experimentelle Pharmakologie als Grundlage der Arzneibehandlung, 3 Aufl., 1914.
- (8) TASHIRO, S.: Amer. Journ. of Physiol., xxxii, 1913.
- (9) TASHIRO, S.: A Chemical sign of life. Chicago, 1917.
- (10) TASHIRO, S., and ADAMS, H. S.: Intern. Zeitschr. f. physik-chem. Biol., i, 1914.

- (11) TRAUBE, J.: Arch. f. d. ges. Physiol., cliii, 1913.
- (12) TRAUBE, J.: Arch. f. d. ges. Physiol., clx, 1915.
- (13) VERWORN, M.: Deutsche med. Wochenschr., Nr. 37, 190.
- (14) WARBURG, O.: Zeitschr. f. physiol. Chem., lxvi, 1910.
- (15) WINTERSTEIN, H.: Zeitschr. f. allg. Physiol., v, 1905
- (16) WINTERSTEIN, H.: Biochem. Zeitschr., li, 1913.
- (17) WINTERSTEIN, H.: Biochem. Zeitschr., lxi, 1914.
- (18) WINTERSTEIN, H.: Biochem. Zeitschr., lxx, 1915.

p. ACETYL-AMIDO-ETHOXY BENZENE

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p. Acetyl-amido-ethoxy benzene¹ has been prepared in the pure state by Dr. J. Tcherniac, who has been good enough to supply me with sufficient for a biological investigation. It occurs as a micro-crystalline powder or as crystals which crystallize from warm water in rhombic or oblique prisms with a good prismatic cleavage.² It is of a colour which just fails to be pure white, and is of a silky appearance. It is odourless and possesses a faintly bitter taste. Its solubility in water is as follows:

	parts in 100 parts of water
At 0°C.....	1.14
At 10°C.....	1.32
At 15°C.....	1.60
At 20°C.....	1.62
At 30°C.....	2.50
At 40°C.....	3.40
At 50°C.....	9.20

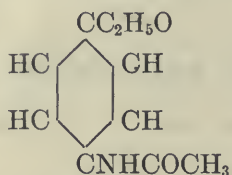
It is readily soluble in alcohol. Its melting point is approximately 120°C.

The similarity of its chemical constitution to that of phenacetin suggested the advisability of comparing the pharma-

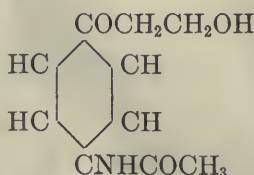
¹ *p*-Acetyl-amido-ethoxy benzene has been put on the market under the name of "Pertonal." For the sake of brevity this name will be used hereafter in this communication.

² I am indebted to Mr. A. Hutchinson for this description of the form of the crystal.

cological actions of the two bodies under the same conditions, and as far as possible this has been done.³ The fact that phe-



Phenacetin.



Pertonal.

nacetin is soluble only to the extent of about 1 in 1700 in cold water precludes the possibility of carrying out this comparison throughout, as for example where an intravenous injection of a large dose is involved.

GENERAL ACTION ON INTACT ANIMALS

Frogs, rabbits and cats were used. The drugs were administered by mouth, by subcutaneous or by intravenous injection.

A subcutaneous injection of 0.0005 to 0.0010 gram of Pertonal per 1 gram of body-weight to a frog produces no apparent symptoms. A dose of about 0.0015 gram produces sluggish movements and diminished reflexes. A dose of about 0.0020 gram produces an appearance resembling death, the animal lying motionless on its back with legs extended and with no reflexes obtainable; recovery takes place within twenty-four hours. A dose exceeding 0.0022 gram per 1 gram of body-weight kills the frog after an interval varying inversely with the dose (0.0027 gram within twelve hours: 0.0037 gram in four and one-half hours, 0.015 gram in two hours). After the injection of a lethal dose the frog shows a train of symptoms gradually leading up to paralysis and death: at first there may be a period of increased irritability which is followed by depression of reflexes and of movements generally without at first any impairment of volition, the animal avoiding obstacles in the normal way. Soon the power of avoiding obstacles is lost and this in turn is followed

³ The phenacetin used was the product of Bayer & Co. and fulfilled the B. P. Codex tests for purity.

by absence of voluntary movements of all kinds, though at this stage the frog will respond to appropriate stimuli by sluggish movements. Soon after this the animal lies on its back completely paralyzed, remaining in this position until it dies. After death the heart is as a rule found to be contracted, and the leg muscles respond to direct electric stimulation or to stimulation of the spinal cord.

On cats and rabbits doses of less than about 1 gram per kilogram produce no apparent symptoms. After doses varying between 1 gram and 2 grams per kilogram the animals become drowsy: this is the only noticeable symptom with the smaller doses of this range: later there is a period of increased reflex irritability with dilated pupils—sometimes salivation, sneezing and vomiting—again a period of drowsiness, followed by complete recovery usually within twenty-four to thirty-six hours. In larger doses (2 grams and upwards per kilogram of body-weight) similar symptoms are produced with in addition staggering gait, violent shivering, prostration, collapse and death from asphyxia.

Compared with a minimal lethal dose of approximately 2 grams of Pertonal per kilogram, phenacetin in dose of approximately 0.75 gram per kilogram in the cat and a somewhat larger dose in the case of the rabbit is sufficient to cause death after a train of symptoms closely resembling those described for Pertonal. Pertonal, then, is very much less toxic than phenacetin.

ANTIPYRETIC ACTION

The antipyretic action of Pertonal was investigated on rabbits which were previously rendered febrile either by puncture of the corpus striatum (1), by subcutaneous injection of distilled water containing the "fever-producing body" of Hort and Penfold (2) or by subcutaneous injection of peptone (3). The method of raising the temperature by puncture of the corpus striatum was found to be by far the most satisfactory. I followed throughout the method described by Gottlieb (4) as amplified by Barbour (5). The puncturing operation was carried out

under ether anaesthesia and with strict aseptic precautions. The animals recovered very rapidly from the operation and showed no untoward effects: symptoms referable to injury of the nodus cursorius, which Gottlieb describes as of frequent occurrence, I have never noted. The animals reacted to the puncture by a rise in temperature ranging between 104.5° and 106°F. which lasted considerably more than twenty-four hours after the puncture was made. The chief disadvantage of the method of injecting distilled water was that the subsequent febrile period was too short. Subcutaneous injection of peptone (Witte's) in doses of 1 gram per kilogram was found to raise the temperature but slightly, rarely above 103.3°F. , the point stated by Hort and Penfold as the result of a large number of observations to be the normal upper limit of the rabbit's body-temperature.

As a matter of routine it was found convenient to perform the puncture operation in the afternoon of one day and to test the action of the antipyretic during the following day. As a rule three animals were used in each batch, all punctured at the same time (within one hour), and on the following day of these three one was treated with Pertonal, one with phenacetin and one kept as a control. The drugs were given either by mouth (through a stomach-tube) or by subcutaneous injection. Ample precautions were taken to ensure accurate thermometry; an ordinary clinical thermometer was used, the same instrument being employed on every occasion. The rectal temperature was taken, the thermometer being inserted to the same distance in every case (up to the 105° mark—about $3\frac{1}{2}$ inches), and the thermometer was held in position for the same period (three minutes) at each reading. The readings were taken as a rule at thirty minute intervals. The rabbits used in these thermometric experiments were all between 1500 grams and 3000 grams in weight. (Hort and Penfold (2) have shown that rabbits of less than 1500 grams are unsuitable, as their body-temperature is very unstable, whilst those larger than 3000 grams are equally unsuitable for this type of experiment, as they are abnormally resistant to factors which tend to produce changes in the body-temperature).

It was found that whilst control animals ran a temperature of 105° to 106° for thirty-six hours or more after the puncture, after which a gradual subsidence to normal took place, those treated with adequate doses of Pertonal or of phenacetin reacted by remissions in the temperature corresponding with the dose which they received. Pertonal in doses up to 0.25 gram per kilogram produces little or no change: in doses of 0.25 to 0.50 this drug reduces the raised temperature by 1.5° or 2° but fails to bring it down to the normal level: in doses above 0.5 gram Pertonal reduces the raised temperature to the normal level. In doses ranging higher than 1.5 grams per kilogram other symptoms are likely to be induced.

Phenacetin was found to have little or no effect in doses of 0.125 gram per kilogram: doses of 0.5 to 0.75 gram reduce the temperature to about the normal, whilst doses of more than 0.8 gram produce untoward symptoms.

Pertonal, then, lowers the abnormally high body-temperature, producing effects comparable with those of phenacetin, the comparative dose being approximately two parts of Pertonal equivalent to one part of phenacetin. It seemed evident too that the remission of temperature produced by Pertonal follows a more gradual curve and lasts rather longer than that produced by a corresponding dose of phenacetin, the curve of which is distinctly more abrupt. Figure 1 shows temperature charts typical of the series.

NARCOTIC ACTION

This action was investigated on larval frogs (tadpoles). Three batches of tadpoles each containing the same number (usually about thirty) were used at a time, and were placed in three separate glass jars each containing the same amount of fresh river-water. One batch was kept as control, to another was added Pertonal and to the third phenacetin in varying concentrations.

It was found that in a concentration of 1 in 5000 little or no change was produced in the movements of the tadpoles by Pertonal, whilst phenacetin in this concentration produced a distinct lethargy which became evident in about thirty minutes

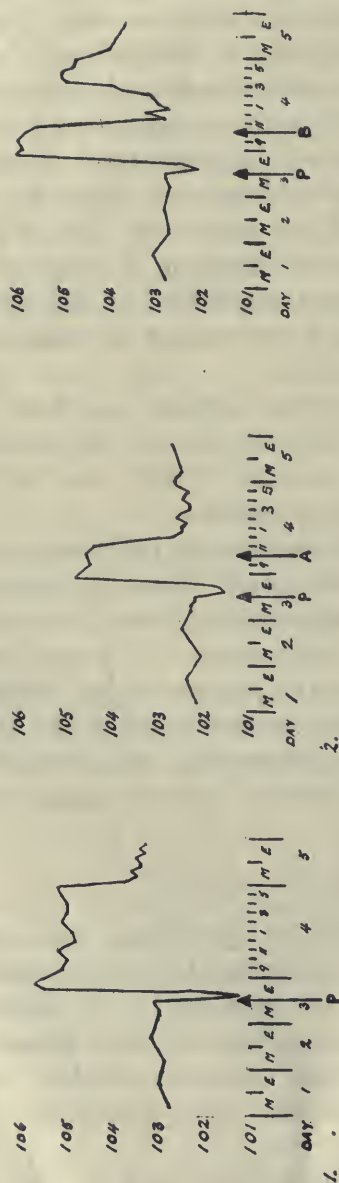


FIG. 1.

1. Rabbit, 2300 grams; P = Puncture of corpus striatum.
2. Rabbit, 2000 grams; P = Puncture of corpus striatum; A = 2 grams Pertonal by stomach-tube (= 1 gram per kilo).
3. Rabbit, 2500 grams; P = Puncture of corpus striatum; B = 2 grams phenacetin by stomach-tube (= 0.8 gram per kilo).

and which in a further two and one-half hours gave place to complete coma. A concentration of 1 in 1000 of Pertonal produced in about two hours distinct lethargy of movements without coma, from which the tadpoles gradually recovered. In concentration of 1 in 200 Pertonal produces lethargy in twenty minutes and coma in three hours, from which complete recovery takes place within a few hours of removal to fresh water. To produce an effect comparable with that of 1 in 200 of Pertonal immersion in a concentration of only 1 in 3000 of phenacetin is required. When two batches of tadpoles are placed one in 1 in 200 Pertonal and one in 1 in 3000 phenacetin, both batches become lethargic and later comatose at the same time and approximately to the same degree: but when changed to fresh river-water recovery is very much more rapid in the tadpoles from the phenacetin solution than in those from the Pertonal (forty minutes as against two hours). When saturated solutions of the two drugs are used, the tadpoles in Pertonal (1 in 60) become lethargic in about fifteen minutes and comatose in about one hour, whilst those in phenacetin (1 in 1700) become comatose in about five minutes. On changing to fresh water complete recovery takes place in the tadpoles from the phenacetin solution in about fifty minutes, whilst of those from the Pertonal only a few recover, and that not until after the lapse of some hours.

It appears, then, that whilst the antipyretic activity of Pertonal is weight for weight about half that of phenacetin, the ratio of the narcotic powers of the same drugs is about as 1 to 15.

FATE OF PERTONAL IN THE BODY

Hinsberg and Treupel (6) investigating certain anilin derivatives found that those which break up in the body with liberation of *p*.amidophenol possess antipyretic and analgesic properties, whilst similar bodies, such as *p*.ethylacetamidophenol, which do not set free *p*.amidophenol, do not possess either antipyretic or analgesic actions. In conformity with this statement one finds that Pertonal sets free in the tissues *p*.amidophenol

and phenetidin. The method of investigation depended on the colour reactions described by Gregoire and Hendrich (7) for *p*.amidophenol and by Edelfson (8) for phenetidin. Rough quantitative estimations were made by comparing the depth of colour produced with certain standard colours produced by equal parts of the same reagents on known concentrations of pure *p*.amidophenol and of phenetidin, which Dr. Tcherniac was kind enough to prepare for me for this purpose. These colour tests were applied to urines and other body-fluids from animals which had received different doses of Pertonal and of phenacetin. It has been claimed for the colour test for *p*.amidophenol that it will distinguish the presence of this body in the urine in concentration as low as 1 in 100000. In my experience, using solutions of pure *p*.amidophenol, there is no certainty in the reaction when the concentration is much lower than 1 in 10000, especially in the case of a urine or any fluid containing pigments which tend to mask the colour reaction aimed at.

It was found that the urine of cats and rabbits which received by the mouth Pertonal in doses of 0.25 gram per kilogram and under, gave a positive reaction for phenetidin but gave only a doubtful *p*.amidophenol reaction. When the dose of Pertonal is increased to about 1 gram per kilogram the urine gives a strongly positive phenetidin reaction and a well marked *p*.amidophenol reaction (equivalent approximately to concentrations of 1 in 2000 and 1 in 8000 respectively). In the case of phenacetin, if sufficient of the drug is given to produce a definite reaction in the urine for phenetidin, the *p*.amidophenol reaction appears to be always positive too.

As regards the length of time over which these bodies are present in the urine after a single dose of Pertonal or of phenacetin it appears that after Pertonal the excretion lasts longer than after phenacetin: this is made clear from the appended protocol taken from one of these experiments which is typical of the series:

DATE	CAT (2500 GRAMS)	CAT (3200 GRAMS)
May 8, 1918	<i>Urine to 9 a.m.</i> phenetidin— <i>p</i> . amidophenol—	<i>Urine to 9 a.m.</i> phenetidin— <i>p</i> . amidophenol—
11.30	1.25 grams Pertonal by stomach-tube (0.5 gram per kilogram)	1.6 grams phenacetin by stomach-tube (0.5 gram per kilogram)
1.00	<i>Passed urine</i> phenetidin + <i>p</i> . amidophenol ? +	
1.30		<i>Passed urine</i> phenetidin + <i>p</i> . amidophenol +
4.00	<i>Passed urine</i> phenetidin + <i>p</i> . amidophenol +	
6.00		<i>Passed urine</i> phenetidin + <i>p</i> . amidophenol +
May 9, 1918	<i>Urine to 9 a.m.</i> phenetidin + <i>p</i> . amidophenol +	<i>Urine to 9 a.m.</i> phenetidin faint + <i>p</i> . amidophenol faint +
	<i>Urine to 3 p.m.</i> phenetidin + <i>p</i> . amidophenol +	
May 10, 1918	<i>Urine to 10 a.m.</i> phenetidin + <i>p</i> . amidophenol +	<i>Urine to 10 a.m.</i> phenetidin ? + <i>p</i> . amidophenol faint +
	<i>Urine to 12.30 p.m.</i> phenetidin + <i>p</i> . amidophenol +	<i>Urine to 12.30 p.m.</i> phenetidin — <i>p</i> . amidophenol —
May 11, 1918	<i>Urine to 10 a.m.</i> phenetidin + <i>p</i> . amidophenol ? +	<i>Urine to 10 a.m.</i> phenetidin — <i>p</i> . amidophenol —
	<i>Urine to 3.30 p.m.</i> Phenetidin — <i>p</i> . amidophenol —	

Other body-fluids tested at various times revealed the presence of *p*.amidophenol or of phenetidin in the saliva (after 1 gram per kilogram of Pertonal to a cat) and in the vomit (after 2 grams per kilogram to a cat).

From a consideration of the structural formula of Pertonal it would appear possible that the drug might be broken up in the tissues with the eventual formation of oxalic acid, which by combination with the tissue calcium would produce untoward symptoms. The probability is however that such formation of oxalic acid could be but slight if any. In any case the intermediate product, ethylene glycol, in the course of its oxidation to form oxalic acid would pass through further intermediate changes with the formation of glycolaldehyde, glycolic acid, glyoxal and glyoxalic acid. Further these glycols have been shown to be harmless to the animal body (9).

In order to confirm the idea that the formation of oxalic acid in the body from Pertonal was unlikely, three experiments were carried out. In each of the first two a pair of rabbits were placed in metabolism cages: one animal of each pair received Pertonal, the others being kept as controls. The doses of Pertonal given were 0.5 gram and 1 gram per kilogram. The urines were collected and allowed to stand in sedimentation tubes, some of the sediment was withdrawn and examined under the microscope for the "envelope" crystals of calcium oxalate. In each case, as it happened, the urine of the control animals contained appreciably more calcium oxalate crystals than did the urines of the rabbits that had received the Pertonal. A further experiment in this connection was performed using a cat, the urine from which was examined for oxalic acid by the method described by Dakin (10). I am greatly indebted to Professor F. G. Hopkins for this estimation, which was carried out under his supervision in his laboratory. A brief protocol of this experiment is appended:

Cat, 1500 grams, under observation in laboratory for seven days, during which time it was fed solely on bread and milk.

April 2, 1918. Placed in metabolism cage.

April 4, 1918. Forty-eight hours urine collected (205 cc. and 160 cc.) Sample A. After collection of sample A, 1.5 grams Pertonal (1 gram per kilogram) fed to animal in bread and milk.

April 6, 1918. Forty-eight hours urine collected (220 cc. and 250 cc.)

Sample B. Samples A and B were then examined for oxalic acid by Dakin's method, which gave the following figures:

365 cc. sample A contains 21 mgm. oxalic acid (0.05 mgm. per cubic centimeter).

470 cc. sample B contains 15 mgm. oxalic acid (0.03 mgm. per cubic centimeter).

It appears then that Pertonal is broken down in the body with the liberation of phenetidin and of *p*.amidophenol: that, as compared with phenacetin, there is a greater tendency to phenetidin formation and a correspondingly smaller tendency to *p*.amidophenol formation: that it is excreted mainly in these forms in the urine: that the breaking down and excretion of Pertonal is somewhat slower and more prolonged than that of phenacetin: that there is no evidence in favour of and all evidence against the possibility of Pertonal giving rise to oxalate formation in the body.

FORMATION OF METHAEMOGLOBIN

Samples of blood were taken from nine animals (cats and rabbits) which had received doses of Pertonal ranging between 0.75 and 2.2 grams per kilogram and from six animals which had received doses of phenacetin ranging between 0.3 and 1.25 grams per kilogram. These samples were subjected to spectroscopic examination. Methaemoglobin absorption bands were found in two cases only: in the blood of cats which had received 1.0 and 1.25 grams of phenacetin per kilogram. No animal which received Pertonal, even in the largest doses employed, showed any trace of methaemoglobin formation.

ACTION ON THE CARDIO-VASCULAR SYSTEM

a. On the heart

The action of Pertonal on the heart was investigated on the intact animal and on the isolated mammalian heart.

Cats were used for the action on the heart of the intact animal: they were anaesthetized first with A. C. E. mixture and

then with Urethane (1 gram per kilogram): a respiration tube connected with the artificial respiration pump was tied into the trachea, a cannula was tied into the jugular vein and the blood-pressure was recorded through a mercury manometer in connection with the carotid artery. The chest was opened and, artificial respiration being carried on continuously, a thread was attached to the apex of the left ventricle and connected over pulleys with a suitably weighted lever which recorded the cardiac

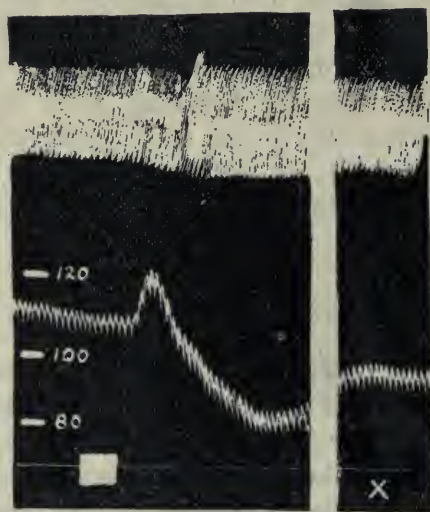


FIG. 2. CAT, URETHANE, HEART MOVEMENTS (DOWNSTROKE = SYSTOLE) AND BLOOD-PRESSURE

At the mark indicated 0.5 gram of Pertonal injected into the jugular vein. X = a portion of the tracing 4 minutes after the injection.

movements on the revolving drum. The effect of an intravenous injection of 0.5 gram of Pertonal in such an experiment is shown in figure 2. A decided increase in the amplitude of the heart's movements, chiefly in the systolic direction, is seen. That this increased amplitude is not entirely a reflex effect dependent on the concomitant vasodilator fall in blood-pressure is shown by the results of experiments in which the isolated hearts of rabbits were perfused through the coronaries by Langendorff's method. In these experiments Ringer-Locke solution

was first perfused for a period of fifteen to twenty minutes until the action of the perfused heart had become steady. Pertonal was then added to the perfusion fluid to make a concentration of 1 in 2000. In control experiments phenacetin was similarly added in the same concentration.

It was found that perfusion of Pertonal in this concentration produced a definite stimulant action on the heart, the effect being most pronounced in the direction of increasing the ampli-

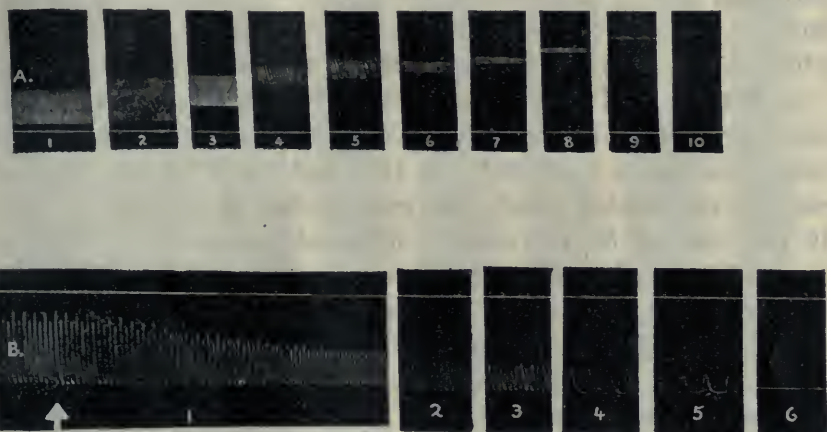


FIG. 3. ISOLATED HEARTS OF RABBITS, PERFUSED LANGENDORFF (UPSTROKE = SYSTOLE)

A, 1 = normal; 2 = 5 minutes after adding Pertonal (1-2000) to the perfusion fluid; 3 = 15 minutes after; 4 = 30 minutes after; 5 = 60 minutes after; 6 = 90 minutes after; 7 = 120 minutes after; 8 = 150 minutes after; 9 = 180 minutes after; 10 = 210 minutes after.

B, 1 = normal and immediate effect of adding phenacetin (1-2000) to the perfusion fluid at the arrow, 2 = 15 minutes after; 3 = 45 minutes after; 4 = 90 minutes after; 5 = 120 minutes after; 6 = 150 minutes after.

tude of the contraction, though in a few experiments there was also some quickening at the same time. Continuous perfusion of Pertonal in this way finally kills the heart in systole in an average time of three hours fifty minutes after adding the drug.

Perfusion of the isolated heart with phenacetin on the other hand produces a depressant effect from the start, the amplitude of the contraction in particular being diminished, and finally kills the heart in diastole on the average three hours ten minutes after adding the drug. Figure 3 shows the effects of the two

drugs apposed for the sake of comparison. It will be seen that Pertonal produces an effect which might be described as a digitalis-like action, whilst the effect of phenacetin is strictly comparable with that of potassium and other cardiac muscle poisons.

b. On the vessels

The action of Pertonal on the vessels was investigated (a) by perfusion of frogs' vessels by the Lawen-Trendelenberg method and (b) by injection into intact anaesthetized cats and dogs, whose blood-pressure and oncometric records were taken.

a. Perfusion of frogs' vessels with Ringer's solution at constant pressure, to which Pertonal was subsequently added, in concentrations of 1 in 1000, 1 in 1500 and 1 in 2000 showed in all cases a vasodilator effect, the rate of flow increasing by from 10 per cent to 50 per cent in different experiments. Control experiments with phenacetin in concentration of 1 in 2000 gave similar results, the rate of flow increasing rather more conspicuously than after similar perfusion with Pertonal.

b. Cats and dogs were used anaesthetized with urethane. In all cases a respiration tube was tied into the trachea and the blood pressure recorded from the carotid artery in the usual way, whilst injections of the drug were given through a cannula tied into the jugular vein. In most cases a loop of intestine isolated with the exception of its blood vessels, which remained intact, and one hind limb were placed in suitable oncometers, and the volume changes of each were recorded with the blood-pressure record. In a few instances the volume of the spleen or of a kidney was taken instead of that of a loop of intestine. In all cases due precautions were taken to ensure that the oncometers were registering accurately: all experiments in which there was reason to think that any extraneous factors came into play were disregarded.

The immediate effect of injecting Pertonal (up to 0.25 gram per kilogram) is a slight fall in blood pressure accompanied by an increase in volume of the internal viscus, the volume of the limb showing little or no change. After larger doses (up to 0.5 gram per kilogram) the immediate fall in blood pressure and

increase in volume of the viscus are more pronounced: the volume of the limb does not as a rule increase at this time. About eight minutes after the injection, at a time when the volume of the viscus and the height of the blood pressure are returning to the normal level, the volume of the limb starts to increase rather rapidly, reaches its maximum in a few minutes, remains high for ten or fifteen minutes, after which it gradually sub-



FIG. 4. DOG, URETHANE, LIMB-VOLUME, INTESTINAL-VOLUME, BLOOD-PRESSURE

A, At the mark indicated 1.5 gram of Pertonal injected into a jugular vein. B = 4 minutes after injection; C = 6 minutes after; D = 10 minutes after; E = 20 minutes after; F = 30 minutes after.

sides. This secondary increase in limb-volume appears to be a constant feature of these experiments: it is more conspicuous in the dog than in the cat and in some instances is most pronounced. The total increase in volume in one experiment was equivalent to 2 cc. in the enclosed portion of the hind limb of a dog weighing 5 kilograms. Figure 4 shows the effect of an injection of 1.5 grams of Pertonal.

This increase in limb-volume appears to be too sharp to be accounted for by a passive dilatation of the vessels secondary to the gradual constriction of the splanchnic vessels or by anything in the nature of oedema. Antipyretic drugs of the anilin class are known to produce their antipyretic action by an increased heat loss, which is brought about by dilatation of surface vessels. The suggestion is put forward that this observed increase of limb-volume is the expression of this effect, and that it does not come on until several minutes after the injection because of the necessity for p.amidophenol or some similar product of the drug being set free before the change can be produced.

ACTION ON RESPIRATION

Cats were the animals used: they were anaesthetized first with A. C. E. mixture and then with urethane (1 gram per kilogram). A respiration tube was tied into the trachea and a cannula into the jugular vein. The blood pressure was recorded from the carotid artery in the usual way, and the respiratory movements by a tambour held rigidly against the chest wall, in connection by rubber tubing with a second tambour which actuated the recording lever. Some slight stimulant effect on respiration was seen to be the effect of Pertonal given intravenously in doses of 0.5 to 1 gram. Whether this effect is due to a direct stimulant action of the drug on the medulla or whether it is secondary to the observed fall in blood pressure was not definitely ascertained.

ANALGESIC ACTION

Little or no information is to be obtained under this head from laboratory experiments. I have taken Pertonal myself on two or three occasions in doses of 15 and 25 grains during neuralgic attacks, which appeared to be relieved by the larger dose.

SUMMARY AND CONCLUSIONS

The pharmacological actions of Pertonal (*p*.acetyl amido ethoxy benzene) have been investigated, and a comparison has been made between it and phenacetin.

Pertonal possesses approximately one-half the toxicity of phenacetin, and as an antipyretic it produces similar effects in doses approximately double those of phenacetin.

Whereas phenacetin is soluble in cold water to the extent of only 1 in 1700, Pertonal is soluble to the extent of about 1 in 60.

Phenacetin exerts a directly depressant action on the heart, which is actually stimulated by Pertonal.

Against an antipyretic ratio of two parts of Pertonal to one part of phenacetin, the corresponding ratio for narcotic action is approximately 15 to 1.

In general the action of Pertonal is less abrupt and more prolonged than that of phenacetin.

Both drugs are excreted mainly in the urine in the form of *p*. amidophenol and phenetidin. It appears that a larger amount of phenetidin and a correspondingly smaller amount of *p*. amidophenol is set free in the tissues by Pertonal than by phenacetin.

No evidence of methaemoglobin formation has been found after Pertonal, whereas this change is often found after phenacetin.

No evidence of oxalic acid formation after Pertonal has been found, though this was specifically sought for.

A range of therapeutic dose of 10 to 20 grains or more is recommended: it is suggested too that the dose need not be repeated so frequently as the dose of phenacetin.

REFERENCES

- (1) ARONSOHN AND SACHS: Pflüger's Archiv., 1885, xxxvii, 232.
- (2) HORT AND PENFOLD: Proc. Roy. Soc. B. 1912, lxxxv, 174.
- (3) KREHL AND MATTHES: Arch. f. exper. Path. u. Pharm., 1896, xxxvi, 437.
- (4) GOTTLIEB: Arch. f. exper. Path. u. Pharm., 1890, xxvi, 419.
- (5) BARBOUR AND WING: Journ. of Pharm. & Exper. Therap., 1913, v, 105.
- (6) HINSBERG AND TREUPEL: Arch. f. exper. Path. u. Pharm. 1894, xxxiii, 216.
- (7) GREGOIRE AND HENDRICH: Bull. de la. Soc. chim. de Belge, xviii, 94.
- (8) EDELFSON: Chem. Centralbl., 1900, i, 573.
- (9) KOBERT: Lehrbuch der Intoxikationen. 2te. Aufl. 1906, 948.
- (10) DAKIN: Journ. of Biol. Chem., 1907, iii, 71.

ON THE PHARMACOLOGICAL ACTION OF ALLOCAIN S. (A NEW LOCAL ANESTHETIC)

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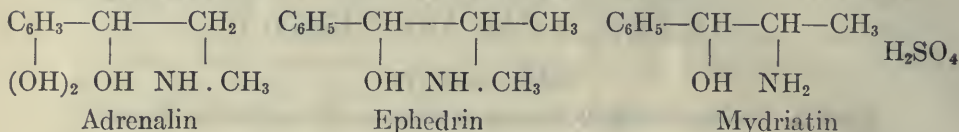
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I. INTRODUCTION

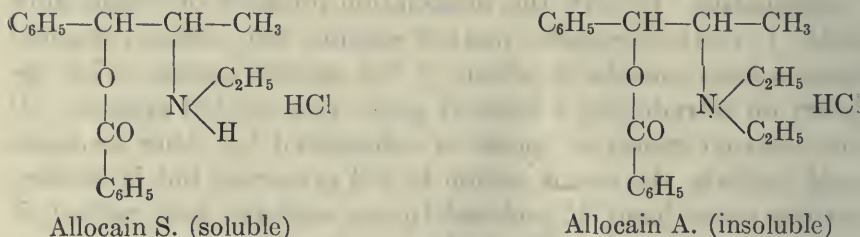
On heating with an alkali, cocain yields ecgonin, methyl alcohol, and benzoic acid. The experiments performed by many investigators (1) with the dissociation products of cocain show that: 1, neither ecgonin, methyl ecgonin nor benzoyl ecgonin have a local anesthetic action; 2, the specific cocain action appears on introducing a benzoyl group into methyl ecgonin. If the benzoyl group of cocain is substituted by other aromatic acid radicals, the cocain action is still preserved but is weaker; on the other hand, if replaced by an aliphatic acid radical, it shows no such action (2); 3, the cocain action appears only on esterifying the benzoyl ecgonin; thus cocaethylin and coca-propylin which are respectively ethyl and propyl esters of benzoyl ecgonin act also like cocain (3). It is, therefore, supposed that the essential factors for the development of the proper action of cocain are: 1, a nitrogenous base like ecgonin; 2, the introduction of an aromatic acid radical such as the benzoyl group in place of its hydrozyl group, and 3, the esterification of its carboxyl group. A number of cocain substitutes have been obtained in this way.

Some years ago, Nagayoshi Nagai (4), (5) succeeded in isolating an alkaloid, ephedrin, from a Chinese drug "*Ephedra vulgaris* Rich. var *helvetica*, Hock et Thomps" which is used as a diaphoretic in the Orient, and afterwards in obtaining a synthetic alkaloid, mydriatin, closely related to ephedrin. These alkaloids have a similar constitution to adrenalin, and according

to the reports of M. Hirose (6), H. Amatsu, and S. Kubota (7), (8) their pharmacological action also resembles qualitatively that of adrenalin. The following formulae show the relationship between these bodies:



More recently the same investigator in conjunction with his assistant S. Kanao, synthesized two new local anesthetics "Allocain S." and "Allocain A." by introducing into mydriatin of mono-ethyl and di-ethyl and benzoyl groups, as shown in the following formulae:



The object of the author was to obtain a body possessing the locally anesthetic properties of cocain on the one hand and a vasoconstricting action like that of adrenalin on the other hand. In the present paper are described experiments concerning the physiological action of Allocain S. as a local anesthetic as compared with that of cocain and novocain, which are the most common local anesthetics in therapeutics at present.

The present author takes this opportunity to express his indebtedness to Professor Doctor Nagayoshi Nagai for furnishing the substance utilized in the experiments.

II. PHYSICAL AND CHEMICAL PROPERTIES

Allocain S. is a white odorless powder consisting of fine needle-like crystals with a bitter and astringent taste, producing on the

tongue a tingling sensation followed by a temporary numbness. It dissolves easily in alcohol and in water with a neutral or a slightly acid reaction.

An aqueous solution of Allocain S. after being acidified with hydrochloric acid produces a white precipitate with corrosive sublimate solution, a brown precipitate with iodine solution, and with potassium hydroxide solution a colorless, transparent, oily liquid which is easily soluble in alcohol and ether. When acidified with nitric acid it yields an abundant white precipitate with silver nitrate solution.

On warming 0.1 gram of the substance with 1 cc. of sulphuric acid for five minutes at about 100°C. and carefully pouring into the resulting solution 2 cc. of water, it deposits on cooling abundant crystals which redissolve in 2 cc. of alcohol.

On rubbing with a glass rod the inside of a test tube containing 1:20 solution of the substance with a half volume of 30 per cent hydrochloric acid, it deposits on cooling needle-like crystals.

These reactions resemble very much those of cocaine.

As Allocain S. is a weak base, it is easily precipitated from solution of its salts in the presence of alkalis. In experimenting with it, it is best to make solutions of it in physiological saline or Ringer's solution without sodium carbonate.

III. PHARMACOLOGICAL ACTION

1. Toxicity

a. *Experiments on frogs.* The smallest quantity of the substance producing any noticeable action upon frogs by injecting into the ventral lymph sac is about 0.00005 gram per gram body weight, and the smallest fatal dose is 0.0004 gram.

The symptoms produced by an injection of poisonous amounts begin with a disturbance of respiratory movements; a slowing of respiration with a growing irregularity and weakness. At the same time there appears also the disturbance of voluntary movements, the animal moving very little spontaneously. When turned on its side it cannot of itself regain the normal posture.

Occasionally a swelling of the abdomen and a trembling of the toes are observed, and mydriasis as well as exophthalmus sometimes appear. In the later stages of poisoning the legs are paralyzed completely and corneal reflexes are weak. On opening the chest, the heart is seen still beating, but it gradually stops. Electrical stimulation of the sciatic nerve and gastrocnemius show that the excitability is apparently not lost.

The results of the experiments with rising doses are arranged in table 1.

TABLE 1

R. esculenta, Allocain *S.* is injected into the ventral lymph sac

EXPERIMENT NUMBER	BODY WEIGHT	SEX	DOSE PER GRAM BODY WEIGHT	RESULT (+ DEAD)	REMARKS
	grams		mgm.		
1	32	♂	0.01	—	No symptoms
2	30	♀	0.01	—	
3	45	♂	0.05	—	Movements somewhat inactive (recovered after about one hour)
4	24	♀	0.05	—	
5	23	♀	0.1	—	Respiration diminished, movements inactive, mydriasis (recovered after five to nine hours)
6	24	♂	0.1	—	
7	19	♀	0.2	—	Paralysis of whole body (recovered before next morning)
8	29	♂	0.2	—	
9	18	♂	0.3	—	
10	23	♂	0.3	—	
11	27	♀	0.4	+	Complete paralysis (dead in thirty-two hours)
12	20	♂	0.4	+	
13	28	♀	0.5	+	Complete paralysis (dead in three hours)
14	26	♂	0.5	+	
15	19	♀	1.0	+	Paralysis (dead in one hour)
16	17	♂	1.0	+	Paralysis (dead in two hours)

b. Experiments on rabbits. Subcutaneous injections of 0.2 to 0.05 grams of the substance per kilogram body weight cause a diminution of spontaneous movements temporarily, with re-

covery in one to three hours. Larger doses (0.4 to 0.5 grams) produce severe convulsions of the whole body, followed by collapse with gradual recovery after some ten hours. After a dose of over 0.6 gram per kilogram body weight, the animal is killed in about ten minutes, after repeated violent convulsions, followed by respiratory failure and collapse.

Experiments with rising doses are summarized in table 2.

TABLE 2

Rabbit, a 10 per cent Allocain S. solution is injected subcutaneously

EXPERIMENT NUMBER	BODY WEIGHT	SEX	DOSE PER KILO-GRAM BODY WEIGHT	RESULT (+ DEAD)	REMARKS
	<i>grams</i>		<i>grams</i>		
1	1100	♀	0.05	—	No change
2	1170	♂	0.1	—	Diminution of spontaneous movements (recovered after one hour)
3	900	♂	0.2	—	Slight paralysis (recovered after three hours)
4	780	♀	0.3	—	Paralysis followed by restlessness (recovered after six hours)
5	800	♀	0.4	—	Paralysis and convulsions (recovered after ten hours)
6	750	♀	0.5	—	Severe convulsions (recovered after twenty hours)
7	1240	♂	0.6	+	Severe convulsions (dead in forty minutes)
8	980	♂	0.7	+	Severe convulsions (dead in seventeen minutes)

A poisonous quantity of Allocain S. therefore causes in frogs a disturbance of respiration as well as of spontaneous and reflex movements, resulting in paralysis of the central nervous system, and in rabbits epileptic convulsions by stimulation of the central nervous system. The lethal dose of Allocain S. for the frog is 0.0004 grams per gram body weight and for the rabbit 0.6 gram per kilogram body weight.

c. A comparison of the lethal dosage of Allocain S. with those of cocain hydrochloride and novocain. According to Biberfeld (9) the lethal dose of cocain hydrochloride is 0.05 to 0.1 gram and that of novocain 0.35 to 0.5 gram per kilogram body weight of rabbit. To make the comparison of lethal dose of Allocain S.

with them more accurate, the author made experiments with cocain and novocain on Manchurian frogs and rabbits.

The results of the author's experiments almost coincided with those of Biberfeld and as is shown in table 3, the lethal doses of Allocain S. for the frog is equal to that of novocain and to four times that of cocain, and for the rabbit to one and a half times that of novocain and four times that of cocain. Thus the toxicity of Allocain S. is considerably weaker than that of cocain and even of novocain.

TABLE 3

SUBSTANCE	LETHAL DOSE FOR	
	Frog	Rabbit
	gram	gram
Allocain S.....	0.4	0.6
Novocain.....	0.4	0.4
Cocain hydrochloride.....	0.1	0.15

2. Anesthetic effect

A. Terminal anesthesia

1. *Anesthesia of frog's skin.* Spinal cord reflex preparations were used. A frog, the brain of which was destroyed and both art. iliacae communes of which were ligated, is suspended by the jaw, and one leg is immersed in a solution of Allocain S. On examination by a weak acid solution, it was found that the application of a 1 per cent solution for five minutes caused a prolongation of the latent reflex period and a diminution of reflex movements. A 5 per cent solution paralyzed the reflex movements in the same period while the control leg which is immersed in the physiological saline solution showed no such anesthetic effect.

2. *Subcutaneous injections in rabbits.* After shaving spots on both sides of a rabbit, in one side 0.5 cc. of allocain solution (1.0, 0.5, or 0.1 per cent) was injected subcutaneously, while in the other side the same amount of physiological saline solution was injected. On stimulating with a needle, it was observed

that on the allocain side there was first a diminution of reflex movements and later no reflex response. In experiments with a 1 per cent solution, an anesthetic effect appeared in five or seven minutes after injection and continued about three hours, and with 0.5 per cent solutions about one and a half hours, but in the experiments with 0.5 per cent solutions, there was only an incomplete anesthesia lasting fifteen to thirty minutes. Such injections, especially of more concentrated solutions were observed sometimes to cause pain to the animal.

3. *Experiments on the tongue of the human being.* On painting 1 or 3 per cent solution of Allocain S. on the tongue, there is produced a bitter taste followed by a paresthesia lasting fifteen to twenty-five minutes, and by using a stronger solution, the sensation of the tongue is almost paralyzed. On the surface of the tongue a white deposit is formed which is due to precipitation of allocain alkaloid, but there remained no destructive or disagreeable effect.

4. *Experiments on the eyes of rabbits.* On instillation of a 1 or 3 per cent Allocain S. solution into the conjunctival sac of rabbits, a slight irritation of the conjunctiva followed by an anesthesia of cornea is produced as early as three or five minutes after the instillation. The cornea could be probed with a sharp point without producing reflex winking of the eye, and no destructive changes in the cornea were noted.

B. Conductive Anesthesia

1. Perineural anesthesia

a. *Experiment on frogs.* 0.3 cc. of an allocain solution was injected into the leg of a spinal cord reflex frog perineurally, and on stimulating the toes by an acid solution, paralysis of reflex movements was observed. In the experiment with 1, 0.5, or 0.1 per cent solutions, there appeared a prolongation of reflex time and a diminution of reflex movements followed by complete anesthesia, but in the experiments with a 0.05 per cent solution, the anesthesia was not complete.

(1) *Paralysis of sensory nerve fibres.* The sciatic nerve of a spinal frog was cut near the knee and it was found that after placing a little ball of cotton soaked with a 1 per cent solution of Allocain S. on the nerve, stimulation of the nerve peripherally to the block produced in three to seven minutes after the application no reflex movements, while in the control side where the same amount of physiological saline solution was applied in the same way no such effect was produced.

(2) *Paralysis of motor nerve fibres.* In nerve-muscle preparations of frogs, it was found after application of a 1 per cent allocain solution to the nerve that the conduction was gradually paralyzed.

Allocain S. has, therefore, a paralytic action upon the conduction of both sensory and motor nerve fibres; the action upon sensory nerve appearing quickly even with a weak solution, while the effect upon motor nerves develops very slowly, sometimes taking over thirty minutes to paralyze the centrifugal conduction of the nerve completely.

b. *Experiments on rabbits.* A short stretch of sciatic nerve of a rabbit was dissected and 0.5 cc. of an allocain solution was injected perineurally.

On stimulating the tip of the toe with a needle it was observed that the reflex movements were lost completely. In the experiments with a 1 and 0.5 per cent solution, anesthesia appeared in three to ten minutes and lasted thirty minutes to 1 hour, but in experiments with a 0.1 per cent solution, the paralysis of reflex movements was not complete.

2. Sacral anesthesia

A rabbit was fixed to a board, and was then injected with 0.3 cc. of a 0.5 or 1 per cent allocain solution subarachnoidally, passing the needle between V and VI sacral vertebrae. The hind paws were paralyzed completely during one or three hours while no untoward effects were noted. In the control animal, in which the same volume of physiological saline solution

was injected in the same way, no such paralytic effect was produced.

3. Comparison of the anesthetic power of Allocain S. with those of cocain and novocain

To get an idea of the anesthetic strength of Allocain S. as compared with cocain and novocain the following experiments were performed:

Solutions of Allocain S. were injected under the skin of a rabbit on one side and cocain or novocain on the other. The time required to paralyze reflex movements was shortest for cocain, while for Allocain S. and novocain it was the same. These differences are demonstrated best by using a weak solution of the substances. As to the duration of anesthesia, cocain was the longest lasting and novocain the shortest lasting of the three.

The following protocols will serve as illustrations:

Experiment 1.—Rabbit, 900 gram, ♀. Comparison of anesthetic power of Allocain S. with that of cocain hydrochloride

TIME	LEFT	RIGHT
Before.....	Reflexes prompt	Reflexes prompt
Injection.....	0.5 cc. of 1 per cent cocain hydrochloride solution	0.5 cc. of 1 per cent allocain solution
After 3 minutes.....	No reflex response	Weak reflex
After 5 minutes.....	No reflex response	Weak reflex
After 10 minutes.....	No reflex response	No reflex response
After 15 minutes.....	No reflex response	No reflex response
After 30 minutes.....	No reflex response	No reflex response
After 60 minutes.....	No reflex response	No reflex response
After 70 minutes.....	No reflex response	No reflex response
After 80 minutes.....	No reflex response	No reflex response
After 95 minutes.....	No reflex response	Weak reflexes appeared
After 105 minutes.....	No reflex response	Weak reflexes
After 135 minutes.....	No reflex response	Almost recovered
After 165 minutes.....	Weak reflexes appeared	Recovered entirely
After 190 minutes.....	Growing stronger	
After 210 minutes.....	Recovered entirely	

Experiment 2.—Rabbit, 1035 gram, ♀. Comparison of anesthetic power of Allocain S. with that of novocain

TIME	LEFT	RIGHT
Before.....	Reflexes prompt	Reflexes prompt
Injection.....	0.5 cc. of 1 per cent novocain solution	0.5 cc. of 1 per cent allocain solution
After 3 minutes.....	No reflex response	No reflex response
After 10 minutes.....	No reflex response	No reflex response
After 15 minutes.....	No reflex response	No reflex response
After 45 minutes.....	No reflex response	No reflex response
After 75 minutes.....	No reflex response	No reflex response
After 90 minutes.....	Feeble reflexes appeared	No reflex response
After 135 minutes.....	Reflexes growing stronger	No reflex response
After 160 minutes.....	Almost recovered	No reflex response
After 190 minutes.....	Recovered entirely	Feeble reflexes appeared
After 210 minutes.....		Weak reflexes
After 240 minutes.....		Getting stronger
After 300 minutes.....		Recovered entirely

In respect to conductive anesthesia, it was found that by using the methods described above that the action of Allocain S. was stronger than that of novocain and weaker than that of cocain. The results of the comparative study showed that the anesthetic power of Allocain is stronger than that of novocain and weaker than that of cocain.

4. Action on blood vessels

The constricting action upon blood vessels is a very desirable quality for a local anesthetic to have, as it delays the absorption of the drug, lessens the possibility of intoxication, prolongs the duration of anesthesia, and lessens bleeding. According to the recent report of Ando (16), the dilator action of cocain and novocain is followed by the constriction of blood vessels while all other cocain substitutes examined by him dilate vessels, and when combined with adrenalin reduce the constricting action of adrenalin. The author studies the action of Allocain S. on blood vessels as follows:

a. Perfusion of the hind legs of toads. The method (13) of Loewen and Trendelenburg was employed and showed that the

outflow from veins increases at first temporarily but is followed soon by a secondary decrease (see table 4).

TABLE 4

Perfusion of toad's hind legs; standard solution, 0.65 per cent saline solution. Test solution, 0.1 to 0.2 per cent allocain standard solution. Pressure of perfusion, 20 cm. H O.

TIME	PERFUSING FLUID	NUMBER OF DROPS IN ONE MINUTE					
		I	II	III	IV	V	VI
<i>minutes</i>							
0	Standard solution...	66	56	79	67	42	72
10	Standard solution...	68	58	80	68	40	72
20	Standard solution...	67	62	81	68	40	70
30	Standard solution...	66	64	80	68	38	70
31	Test solution.....	(0.1%)	(0.1%)	(0.1%)	(0.1%)	(0.2%)	(0.2%)
32	Test solution.....	70	62	82	70	44	80
34	Test solution.....	68	48	78	70	42	68
36	Test solution.....	62	28	70	67	32	57
37	Standard solution...	—	—	—	—	—	—
40	Standard solution...	58	27	62	64	22	40
46	Standard solution...	57	36	60	62	24	42
50	Standard solution...	56	39	62	61	28	44
55	Standard solution...	56	42	64	58	30	46
60	Standard solution...	56	46	66	56	30	48

b. Perfusion of rabbit's ear. Pissemiski's method (11) of perfusion of rabbit's ear was employed. The drop-flow from the veins was recorded on smoked paper by the aid of drop-counter of the author's own construction (10) (see table 5).

As it is seen in the table, at the beginning of perfusion of Allocain S. the number of drops increases markedly but after the perfusion of standard solution it diminishes beyond its original rate, and in this experiment the dilator action appeared more marked than the constrictor.

c. Perfusion of rabbit's kidney. A rabbit's kidney was perfused by the method of Kobert and Thompson (12). The results of the experiments coincided almost exactly with those found in perfusing the rabbit's ear.

Allocain S. therefore has both a dilator and constrictor action upon blood vessels, and in the cold blooded animals, the

TABLE 5

Perfusion of rabbit's ear: Standard solution, 0.9 per cent saline solution. Test solution, 0.05 to 0.2 per cent allocain standard solution. Pressure of perfusion, 110 mm. Hg.

TIME <i>minutes</i>	PERFUSING FLUID	NUMBER OF DROPS IN ONE MINUTE		
		I	II	III
0	Standard solution.....	108	99	82
5	Standard solution.....	102	99	80
10	Standard solution.....	102	99	82
12	Test solution.....	(0.05%)	(0.01%)	(0.2%)
13	Test solution.....	123	138	84
15	Test solution.....	120	142	82
20	Standard solution.....	102	126	80
25	Standard solution.....	—	—	—
30	Standard solution.....	93	111	74
35	Standard solution.....	90	102	69
40	Standard solution.....	87	99	69
45	Standard solution.....	87	81	69

constrictor action manifests itself more markedly, while in the warm blooded animals the dilator effect appears more marked.

5. Action upon circulation and respiration

a. The heart. The heart action of Allocain S. was studied in curarized frogs, observing the exposed heart or recording the beats by the suspension method. A small amount of allocain such as about one-tenth of lethal dose when injected into lymph sac, first diminishes the rate of heart beat, but soon it is found recovered. The injection of a greater quantity diminishes the rate of beat suddenly, and the contraction becomes incomplete, occasionally with an atrio-ventricular dissociation. Then the systole and diastole become incomplete gradually and first the ventricle, then the auricle stops beating. This action is not counteracted by the administration of atropin. It seems therefore that Allocain S. acts upon the motor apparatus of the heart and on the conductivity of the heart muscle so as to slow the rate of beat and to cause the diminution of contraction.

b. The blood pressure. The blood pressure of a dog anesthetized with urethane (1.5 gram per kilogram body weight) was examined. On injecting a weak solution of Allocain S., there was observed a rise of the blood pressure but the injection of a relatively concentrated solution rapidly caused a lowering of the pressure followed by a gradual rising, which lasted several minutes. This action of Allocain S. has nothing to do with the vagus nerve as is evidenced by cutting the nerve or by paralyzing vagus nerve endings in the heart with atropin. The primary lowering of the blood pressure may be probably due to both

Experiment 3.—Rabbit, 1320 gram, ♀. Injected intravenously with a 0.5 cc. of a 2 per cent allocain solution

TIME	BLOOD PRESSURE	RATE OF HEART BEAT IN ONE MINUTE	NUMBER OF RESPIRA- TIONS IN ONE MINUTE
	mm. Hg.		
Before.....	98.5	264	39
Injection.....	(0.5 cc. of a 2 per cent Allocain S.)	—	—
After 1 minute.....	44.0	234	42
After 2 minutes.....	92.0	245	42
After 4 minutes.....	109.0	228	48
After 6 minutes.....	124.5	116	48
After 12 minutes.....	118.5	234	46
After 18 minutes.....	113.5	246	48
After 33 minutes.....	107.0	246	50
After 38 minutes.....	104.0	246	42
After 42 minutes.....	101.0	252	42

heart and blood vessel action, and the secondary rise of the pressure may be explained partly by the constrictor action upon blood vessels.

c. The respiration. The injection of 0.1 mgm. per gram body weight of frog causes a remarkable disturbance of respiration; a slowing of respiration followed by irregularity and shallowness, and finally by paralysis. As it was observed before, the minimum lethal dose of Allocain S. does not paralyze the muscle or nerve, this action upon respiration must be considered to be the action upon the central nervous system, especially upon respiratory

center. In experiments with warm blooded animals it is hard to estimate the action upon respiration itself separately on account of the convulsions. It is possible that Allocain S. here first stimulates and then paralyzes the center.

6. Bactericidal action

In these experiments, the author used streptococcus and staphylococcus isolated from the sputum of a tubercular patient. The former was cultivated eighteen hours and the latter forty-two hours on agar surface. One loopful of each coccus was taken and placed in sterile saline solution containing Allocain S. for five, ten, and thirty minutes, and the cultures in agar and bouillon were again made. The action of cocain and novocain upon these cocci was studied in the same way as that of Allocain S. On examination of the cultures it was found that 1:20 Allocain solution inhibits the growth of staphylococcus completely in 30 minutes and 1:80 solution of it incompletely, while 1:20 cocain solution can exert only a slight inhibiting effect in the same time, and novocain even after thirty minutes exposure with 1:20 solution shows no action at all. 1:100 Allocain solution can inhibit completely in five minutes the growth of streptococcus while 1:40 cocain solution takes thirty minutes and novocain shows no bactericidal action.

IV. DISCUSSION

The toxicity of Allocain S. is less than that of either cocain or novocain, and it has a more powerful anesthetic action than novocain. Moreover, it possesses a slight bactericidal action and a slight vascular action, and can stand exposure to heat to some degree. Owing to a temporary hydrolytic dissociation it turns turbid, but after cooling returns to its original state and there occurs no change in the anesthetic power. These characteristics of Allocain S. speak in favor of its use as a local anesthetic, but it has on the other hand an undesirable quality.

The Allocain S., being a compound of HCl and a weak base, frees acid easily in its solutions and by adding even a weak

alkali, an insoluble base is precipitated. On applying to mucous membranes, there appears a white deposit which may prevent the further development of anesthesia and on using subcutaneously an irritation may be caused by its acid reaction, and the precipitation of allocain base by tissue fluids is to be expected at the place of injection, causing some disadvantageous effect.

V. SUMMARY

1. Allocain S. causes paralysis in frogs and convulsions in rabbits, acting upon the central nervous system.

2. The lethal dose of Allocain S. when injected subcutaneously is for frogs 0.0004 gram per gram body weight, and for rabbits 0.6 gram per kilogram weight, and is smaller than that of cocain and novocain.

3. Allocain S. causes a local paralysis of the sensory nerve endings and nerve fibres, and its anesthetic power is stronger than novocain and weaker than cocain.

4. Allocain S. has a two-fold action upon blood vessels; a primary dilation and a secondary constriction, and in warm blooded animals the former action, and in cold blooded animals the latter appears more marked.

5. Subcutaneous injections of Allocain S. cause a slight local irritation.

6. A great amount of Allocain S. paralyses the heart, acting upon the motor apparatus and conductive system, and causes in frogs a paralysis of respiration and in rabbits a stimulation of it, which action seems to be central in character.

7. The blood pressure is influenced by Allocain S. The substance produces a primary fall of pressure which is followed by a rise above the normal.

8. Allocain S. inhibits the growth of both strepto- and straphylococci.

9. Allocain S. has a good character as a local anesthetic in many respects, but on the other hand it has also some unfavorable qualities. On account of the slight irritation by its acid solutions and of its precipitation by tissue fluids, its use is limited.

ADDENDUM

Since the Allocain S. has been introduced into therapeutics, it has been tried on several hundred cases of operation with success. The results of clinical experiences noted by many physicians are in many respects in accordance with my experiments on animals. It has indeed a stronger anesthetic and a lesser toxic action than novocain, but it has sometimes a slight irritation at the moment of injection which may be due to the free acid of the solution, and in a few cases a slight necrotic action is observed where it is injected in concentrated solution, or where it is applied several times in the same place. Otherwise no disadvantageous effect is noticed. Recently the discoverer of Allocain claims that he has improved the method of preparing the substance so that its solutions are no longer irritating to the tissues.

REFERENCES

- (1) FRAENKEL, S.: *Arzneimittelsynthese*, 1912, iii Auflage, 351.
- (2) FILEHNE: *Berliner klinische Wochenschrift*, 1887, 107.
- (3) POULSON: *Arch. f. exp. Path. u. Pharm.*, 1890, xxvii, 301.
- (4) NAGAI, N.: A study on a Chinese drug *Ephedra vulgaris*. *Yakugaku Zassi* (Japanese Journal of Pharmacy) 1892, no. cxx, 109; no. cxxi, 180 and 1893; no. cxxxix, 901.
- (5) NAGAI, N.: On the synthesis of ephedrin, an active principle of *ephedra vulgaris*. *Yakugaku Zassi*, 1909, no. cccxxix, 739.
- (6) HIROSE, M.: Ueber die pharmakolog. Eigenschaften einiger dem Adrenalin nahestehender Substanzen, *o*-Dioxyphenylaethanolamin, Phenyl aethanolamin, Ephedrin, Mydriatin. *Mitteilungen aus der Med. Fakult. d. Kaiserl. Universit., (Tokyo)*, 1889, xiii, 2555.
- (7) AMATSU, H., AND KUBOTA, S.: On the pharmacological action of ephedrin and mydriatin. *Kyoto Igaku Zassi*, 1912, x, and 1917, xiv, no. 1.
- (9) BIBERFELD, Y.: *Pharmacologisches ueber Novocain*. *Medezin. Klinik* 1905, Nr. xliix, 1218.
- (10) KUBOTA, S.: On the effect of osmotic pressure on the calibre of blood vessel. *Tokyo Igaku Zassi*, 1913, xxvii, no. xxi.
- (11) PISSEMISKI, S. A.: *Pflueger's Archiv f. d. ges. Physiolog.* 1914, clvi, 426.
- (12) KOBERT AND THOMPSON: *Kobert's Lehrbuch der Intoxication*, 1902, Bd 1, ii Auflage, 171.
- (13) ANDO, S.: *Kyoto Igaku Zassi*, 1915, xii, no. 1, 1.

EFFECT OF ATROPIN ON ETHER HYPERGLYCEMIA

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It is well known that ether anesthesia increases the amount of dextrose in the blood. Excitement (1), asphyxia (2), chloroform anesthesia (3), or morphine (4) will bring on a hyperglycemia. Practically any shock to a normal body, at least temporarily, increases the blood sugar. If by any means the hyperglycemia of ether anesthesia could be reduced, it is hoped that the same means would effect a similar reduction in the shock resulting from anesthesia.

The effect of dieting previous to the anesthetic has been investigated (5). It was found that dogs which had received a pure meat diet were disposed to give a greater blood sugar rise than those getting a mixed diet. Morphine is often given before an anesthetic for the purpose of reducing the excitement or over-tension preceding anesthesia. It was found that this drug did not alter the rise in the amount of dextrose in the blood (4). Atropin is frequently employed in preanesthetic medication for the purpose of reducing secretions and preventing cardiac inhibition. For reasons to be given later, it was thought that atropin would reduce ether hyperglycemia. Therefore the following work was done.

EXPERIMENTAL WORK

Dogs were used as subjects. They were not dieted previous to the experiment. They were not allowed food within sixteen to eighteen hours previous to the test. They were bled by direct puncture of the jugular vein with hypodermic needle and 8 to 10 cc. of blood were taken each time and kept from clotting

with sodium oxalate. The blood dextrose was determined by the method of Benedict (6) except that the picric acid solution contained 0.05 instead of 0.5 per cent sodium hydroxide. Triplicate determinations were made in every case.

A series of seventeen dogs was run to determine the effect of ether anesthesia on blood sugar. Each dog was bled and then given ether by putting the head into an ether can into which air saturated with ether was forced. Fifteen minutes after anesthesia started the dog was bled a second time. The animal was kept under the ether for an hour and bled a third time. The results are given in table 1.

TABLE 1

Effect of ether anesthesia on blood sugar in normal animals

DOG	NORMAL	AFTER ANESTHESIA OF FIFTEEN MINUTES	AFTER ANESTHESIA OF ONE HOUR
1	0.098	0.142	0.114
2	0.101	0.137	0.189
3	0.080	0.132	0.138
4	0.091	0.122	Dog dead
5	0.094	0.133	0.131
6	0.090	0.114	0.087
7	0.121	0.141	Dog dead
8	0.082	0.128	0.118
9	0.081	0.085	0.101
10	0.084	0.171	0.230
11	0.086	0.132	0.177
12	0.072	0.123	0.150
13	0.097	0.117	0.172
14	0.093	0.118	0.144
15	0.086	0.119	0.089
16	0.088	0.105	0.118
17	0.086	0.138	0.156

The second series of animals was run to determine the action of ether anesthesia after the administration of atropin. The dogs were bled, given atropin intravenously, after fifteen minutes bled again, then given ether and bled at same intervals as in previous series. Ether was administered the same way as in the previous series. The results are given in table 2.

TABLE 2

Effect of ether anesthesia on blood sugar in atropinized animals

DOG	NORMAL	AFTER ATROPIN FIFTEEN MINUTES	AFTER ANESTHESIA OF FIFTEEN MINUTES	AFTER ANESTHESIA OF ONE HOUR
1	0.122	0.117	0.130	0.132
2	0.076	0.069	0.079	0.095
3	0.095	0.095	0.121	0.159
4	0.112	0.104	0.124	0.116
5	0.112	0.108	0.126	0.151
6	0.107	0.101	0.114	0.114
7	0.109	0.101	0.103	0.105
8	0.105	0.105	0.123	0.114
9	0.120	0.111	0.137	0.187
10	0.164	0.104	0.103	0.109

A third series of dogs was used to determine the action of atropin alone on the blood sugar. The dogs were bled at the same intervals as in the second series and the same amount of atropin was given. Results are given in table 3.

TABLE 3

Effect of atropin on blood sugar in normal animals

DOG	NORMAL	AFTER ATROPIN FIFTEEN MINUTES	AFTER ATROPIN THIRTY MINUTES	AFTER ATROPIN SEVENTY-FIVE MINUTES
1	0.121	0.119	0.118	0.100
2	0.112	0.105	0.107	0.105
3	0.098	0.099	0.094	0.098
4	0.097	0.094	0.096	0.098
5	0.104	0.105	0.105	0.104
6	0.099	0.101	0.096	0.094
7	0.099	0.100	0.097	0.101
8	0.095	0.092	0.097	0.100
9	0.100	0.101	0.100	0.098
10	0.097	0.097	0.099	0.093

The amount of atropin given was determined by Sollman's (7) recommendation of 0.05 mgm. per kilogram of body weight. This amount should paralyze the vagus. To be sure of doing this the dosage was doubled.

DISCUSSION

In table 1 the effect of ether anesthesia on blood sugar of seventeen dogs is indicated. The results are expressed in per cent of dextrose in blood. There was a wide variation in animals as indicated by the glycemias recorded. However, the action of fifteen minutes of anesthesia was quite constant for all animals. There was a decided increase in all but one case. After an hour of anesthesia there was a hyperglycemia in all but two cases, and

TABLE 4

Average blood sugar findings expressed in per cent of dextrose

	NORMAL	FIFTEEN MIN- UTES ACTION OF ATROPIN	FIFTEEN MIN- UTES ACTION OF ETHER OR ATROPIN OR BOTH	ONE HOUR ACTION OF ETHER OR ATROPIN OR BOTH
Ether alone.....	0.090	None	0.127	0.141
Ether after atropin.....	0.106	0.101	0.116	0.128
Atropin alone.....	0.102	0.101	0.101	0.099

TABLE 5

Average blood sugar findings expressed in per cent of normal blood sugar

	NORMAL	FIFTEEN MIN- UTES ACTION OF ATROPIN	FIFTEEN MIN- UTES ACTION OF ETHER OR ATROPIN OR BOTH	ONE HOUR ACTION OF ETHER OR ATROPIN OR BOTH
Ether alone.....	100	None	141	157*
Ether after atropin.....	100	95	109	121
Atropin alone.....	100	99	99	97

* This is calculated leaving out the normal values of the animals which died before the test was completed.

in all but three cases the blood sugar was higher at the end of the hour than at the end of fifteen minutes of anesthesia. In referring to the table of averages, table 4, it is clear there was a decided increase in blood sugar as a result of ether action. Table 5 indicates that the per cent of increase in the first fifteen minutes was 41 per cent, and in the hour it was increased to 57 per cent. In a previous paper (5) a similar series was recorded but the method of dextrose determination was different so it was thought

best to repeat the work. The results of the two series are in approximate agreement. Before ether, after fifteen minutes and after one hour of anesthesia, the averages were 0.075, 0.117, 0.130 and 0.090, 0.127, 0.141 per cent for the previous and present series respectively. The same averages expressed in per cents of the normal values are 100, 156, 173 and 100, 141, 157. The wide variation in animals and the limited number employed does not warrant close comparison. However, in the light of these results it seems fair to conclude that fifteen minutes of ether anesthesia increases blood sugar 40 to 50 per cent, and one hour of anesthesia increases it from 50 to 75 per cent.

The results in table 2 show the effect of ether on animals which had been given atropin. Sufficient atropin was given to paralyze the cardiac vagus. That animals were affected differently was indicated by their pupils. A very few had only a small amount of pupil dilation. Whether the vagus paralysis is directly proportionate to the size of the pupil, it is impossible to tell. It is thought that some degree of the individual variations was due to the differences in the individual susceptibility to atropin. The dogs were bled and given the atropin sulphate intravenously. After fifteen minutes the dogs were bled again and proceeded with as in the previous series. At the end of fifteen minutes of anesthesia the blood sugar was increased over the normal findings in eight cases out of ten, but the increases were not nearly as large as those without atropin. Table 4 shows that the normal in this series of animals was 0.106 per cent and after fifteen minutes of anesthesia the blood sugar was 0.116 per cent. There was an increase of 9 per cent above normal. An hour of anesthesia produced some degree of hyperglycemia in all but one animal. The increases as a rule were smaller than without atropin. This is shown in tables 4 and 5 which indicate that the final average was 0.128 per cent dextrose in blood or an increase above the normal of 21 per cent.

The results of the third series of animals are given in table 3. The purpose of this group of tests is to determine whether the amount of atropin used produced any change in blood sugar, and the amount of same if such did occur. The amount of

atropin used was the same as with the second series of animals, and the times of bleeding were also the same. It will be noticed that the individual variation was about the same as usual but the variation of the glycemia of each individual was small. The average given in table 4 indicates practically no effect on blood sugar. The per cent changes given in table 5 are negligible.

Summing up the results obtained, it was found that 15 minutes of anesthesia without atropin caused a glycemia of 41 per cent above normal and with atropin, a glycemia of 9 per cent above normal. The atropin caused a reduction of 79 per cent in the increase due to fifteen minutes of ether anesthesia. An hour of anesthesia increased the blood sugar 57 per cent without atropin and 21 per cent with atropin. Here, as in the case of the fifteen minute period, there seems to be much in favor of the atropin medicated dogs. However, when it is noted that in the last forty-five minutes the increase without atropin was 16 per cent and with atropin 12 per cent, one is forced to conclude that after the first fifteen minutes of anesthesia the atropin accomplishes little. Therefore it seems safe to conclude that atropin materially reduces the hyperglycemia resulting from ether anesthesia and that this is accomplished mainly in the first fifteen minutes of the drug's action.

The mechanism of the action of atropin in preventing the regular hyperglycemia of ether anesthesia, is uncertain. Morat, Cavazznai and Soldani (8) credit atropin with the power to reduce the ease with which glycogen is broken up in the liver. Macleod by work in 1908 disagrees with their conclusions (9). Rudisch (10) and Forcheimer (11) claimed that atropin increases the tolerance of diabetics for sugar. They used large doses of atropin and succeeded in the majority of cases in reducing the sugar output. Mosenthal (12) reported two cases in which he was unable to bring about such a reduction with atropin. In a private communication, Dr. F. C. Becht states that such a reduction is to be expected, not for the reason of altered metabolism, but because of reduced digestion of carbohydrates resulting from reduced secretion of digestive fluids.

Thus, there are three possible explanations of the fact that atropin reduces the rise in the blood sugar due to ether anesthesia: first, altered metabolism; second, reduced cardiac inhibition; and third, reduced respiratory inhibition. That the last two factors accomplish much in this direction seems probable. However, more work must be done on this phase of the subject before final conclusions can be drawn.

The use of atropin clinically or in animal experimentation adds difficulties and dangers. These were realized in this piece of work. A number of dogs died. Two guides for the anesthetist are modified. The pupil reaction is destroyed. The respiration is stimulated so that its rate and depth are reduced at a much deeper stage of anesthesia than when atropin is not used. The mouth and throat get so dry that the tongue and epiglottis often plug the respiratory passages.

SUMMARY AND CONCLUSION

The purpose of this work was to determine the effect of atropin on hyperglycemia from ether anesthesia. Three series of animals were observed. The first series consisted of seventeen dogs. They were kept under ether anesthesia one hour and were bled just before ether was administered, fifteen minutes after anesthesia started, and at the end of one hour of anesthesia. The second series, consisting of ten dogs, was tested in exactly the same manner except that fifteen minutes before anesthesia they were bled and given atropin intravenously. The third series of ten dogs was treated just like the second series except that no ether was given. Ether anesthesia raised the blood sugar in fifteen minutes 41 per cent without atropin, and 9 per cent with atropin. An hour of anesthesia raised blood sugar 57 per cent without atropin, and 21 per cent with atropin. Atropin alone did not change the glycemia appreciably.

The results lead to the following conclusions: First, atropin reduces markedly ether hyperglycemia. Second, the greatest reduction in ether hyperglycemia brought about by atropin is in the first fifteen minutes.

REFERENCES

- (1) SHAFFER, P. A.: J. Biol. Chem., xix, 302, 1914.
- (2) MACLEOD, J. J. R.: Diabetes, p. 178. Longmans, Green and Co., 1913.
- (3) SCOTT, E. L.: Amer. J. Physiol., xxxiv, 271.
- (4) ROSS, E. L.: J. Biol. Chem., xxxiv, 335, 1918.
- (5) ROSS, E. L., AND MCGUIGAN, H.: J. Biol. Chem., xxii, 407, 1915.
- (6) BENEDICT, S. R.: J. Biol. Chem., xxxiv, 203, 1918.
- (7) PILCHER, J. D., AND SOLLMANN, J.: Pharmacol., v, 317, 1914.
- (8) SOLLMANN, T.: Manual of Pharmacology, p. 288, W. B. Saunders Co., 1917.
- (9) MACLEOD, J. J. R.: Proc. Soc. Exp. Biol. and Med., v, 90, 1908.
- (10) RUDISCH, J.: J. Amer. Med. Assn., liii, 1366, 1909.
- (11) FORCHEIMER, F.: Amer. J. Med. Sc., cxli, 157, 1911.
- (12) MOSENTHAL, H. O.: J. Amer. Med. Assn., lviii, 777, 1912.
- (13) CUSHNY: Pharmacology and Ther., p. 161, Lea and Febiger, 1913.
- (14) MEYER AND GOTTLIEB: Pharmacology, p. 59, Lippincott, 1914.
- (15) HARE H. A.: Practical Therapeutics, p. 268, Lea and Febiger, 1918.

AN APPARATUS FOR THE ADMINISTRATION OF GASES AND VAPORS TO ANIMALS

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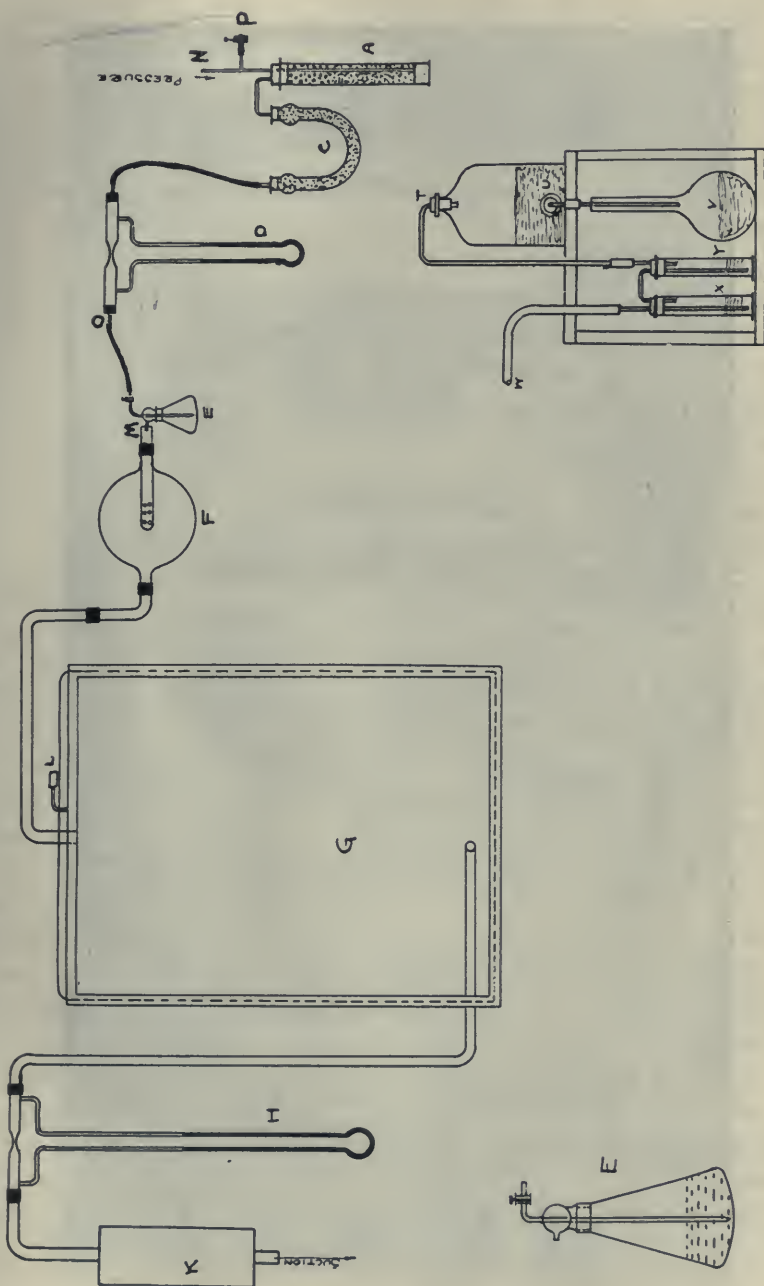
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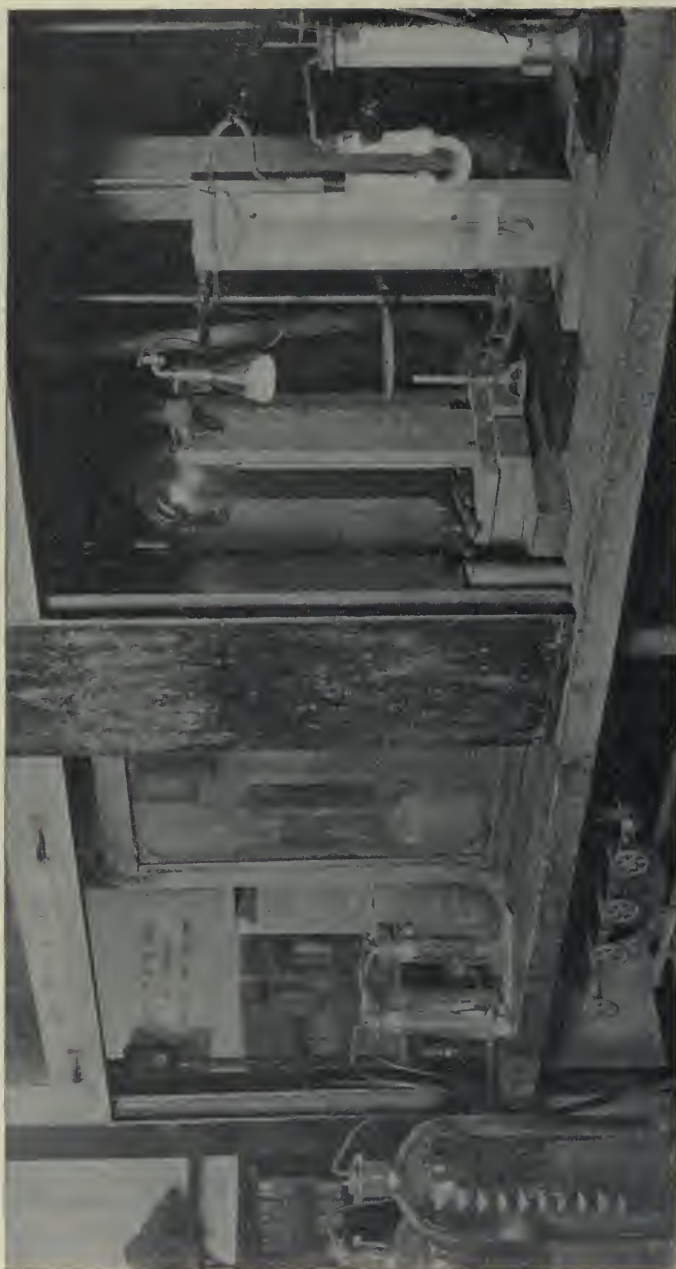
The apparatus illustrated in the accompanying photograph and diagram has been used now for over a year in various investigations on poisonous gases.¹ Most of the investigations which have appeared in the literature on the poisonous action of various gases and vapors are inaccurate in the matter of dosage, as precautions to maintain a constant amount of the poison in the atmosphere and to determine the exact concentrations used, were neglected. The animals were usually placed in a closed bell jar or box, and a certain amount of the substance introduced. The absorption by the box and hair and skin of the animal, as well as incomplete vaporization when liquids were sprayed, introduces a grave error which can only be partially overcome by actual analysis of the contents of the chamber. This is due to the fact that the concentration is continually decreasing due to absorption. In a long series of investigations extending from 1884 to 1913, Lehmann studied the effects upon animals of various poisonous gases used in the industries (1). The method described in this paper is based upon the method used by Lehmann and his pupils, but has been modified and simplified sufficiently to warrant description. Lehmann's method consisted in exposing the animal in a modified Petenhoffer respiration apparatus (2) to a continuous flow of air containing a constant and known concentration of the gas. The concentration of gas employed was determined by

¹ This apparatus was devised in New Haven, Connecticut, in the early summer of 1917, when the war gas investigations were started in this country; under the direction of the Bureau of Mines.

analysis of samples of air drawn from the chamber. Some modification of the apparatus was subsequently made. The differences and modifications of the present apparatus can be seen by a reference to the various illustrations in Lehmann's articles (3).

The accompanying diagram and photograph show the details of the apparatus. *G* is an air-tight, glass box 20 by 25 by 15 inches and of about 130 liters capacity, with a sliding, plate glass door that can be made air-tight with adhesive tape and plastecine after closure. The plates of glass are ground to fit tightly together in a wooden frame, bolted in, and sealed with white lead between the glass and inside of the wooden frame, and putty on the outer edges of the frame. A current of air enters the chamber in front on the top just behind the door, and leaves at the back and bottom. The outlet which is most convenient to prevent breakage or stoppage by the animal, is an iron elbow, which is connected with the glass tubing beyond the box. *L* is an opening for obtaining samples while the apparatus is in operation. *F* is a mixing chamber of glass of about 3 to 4 liters' capacity. *D* and *H* are flowmeters (venturi meters) calibrated against a standard water meter. *H* is made so as to record 250 liters per minute. *D* is most conveniently of three or four flowmeters which can measure the range between 1 cc. and 20 liters per minute. They are used for measuring air flow. *K* is a metal can filled with charcoal and soda lime to absorb the gas in the exit air before it passes through the pump. *E* is a specially designed flask of about 200 cc. capacity for containing the liquids to be vaporized. *A* and *C* are calcium chloride tubes for drying the air, which subsequently passes through the liquid. The connections of the chamber are made of glass tubing 1 to 1½ inches in diameter. The tube at *N* is attached to a "blower" for delivering compressed air. (Any small positive blower can be used for this purpose). The small Eimer and Amend blower no. 1 has been used. The outlet of the absorber *K* is connected to an apparatus for obtaining suction. Capacity, rather than any great degree of negative pressure, is desired. We have used a Crowell pump no. 3, capacity 25 to 28 cubic feet. of free air per minute.





The manipulation of the apparatus is briefly as follows: Air is drawn in at *M*; and through the chamber *G* at the rate of 250 liters per minute. This is measured by the flowmeter *H*. If the substance to be investigated is a gas at ordinary temperatures and pressure, it is used in liquefied form in a steel cylinder or is collected in a gasometer. The flask *E* is removed and the end *O* of the flowmeter *D* placed at the opening in the mixing chamber *M*. The gas is passed at a rate to obtain the desired concentration into the mixing chamber *F*, where it is greatly diluted with pure air. The rate of flow is measured by the meter *D*. On the other hand if a liquid is to be vaporized and investigated, about 50 to 75 cc. is placed in the flask *E*. The upper parts of the flask are filled loosely with glass wool to prevent droplets being carried into the mixer. The flask *E* is carefully weighed and connected to the apparatus. Dry air is then blown at a constant rate through the liquid in *E*, thereby vaporizing a portion into the mixer *F*. The concentration is regulated by the rate of air per minute passed through the liquid in *E*, and is controlled by the flowmeter *D*. This flowmeter is calibrated for a wide range of rates of flow, or better, 2 or 3 meters with different ranges of flow are used. The rate of flow through the liquid necessary to obtain a desired concentration varies greatly with the vapor pressure of the liquid. Another way of regulating the concentration is to keep the air flow through *E* constant, and vary the temperature of the flask *E* by immersing in a warm or cold bath. By using an ice bath, one can work with liquids which do not boil lower than about 10°C.

The concentration in the case of a gas can be roughly calculated from the relative flows through the flowmeters *D* and *H*. It can be accurately determined in any case by analysis of samples drawn from the chamber through the tube *L*. A convenient apparatus for sampling is shown in the diagram. *X* and *Y* are bottles filled with an appropriate absorbing solution for the gas. *W* is connected to *L*, and water withdrawn from the large bottle at *U* into a graduated vessel *V*. The size of the sample is determined by the amount of water delivered into *V*. In the case of liquids when the flask *E* is used, this flask is weighed

accurately before and after the experiment. The loss in weight gives the total quantity of the liquid vaporized. Since 250 liters per minute are passing through the chamber, knowing the time the apparatus has been in operation, the concentration can easily be calculated. For example, with an exposure of thirty minutes and flow of 250 liters, 7500 liters have passed through the apparatus. Suppose the flask *E* lost 7.5 grams in weight. Then the

concentration in the chamber is equal to $\frac{7500 \text{ mgm.}}{7500} = 1 \text{ mgm. per liter}$. This "loss in weight" method is very simple and much more convenient than analytical control by sampling from the chamber *G*. A similar "loss in weight" method of determining concentration was used by Lehmann in his work on turpentine and heavy hydrocarbon oils (4). Provided the sample of liquid used is a very pure homogenous sample, the "loss in weight" method gives as accurate results as can be obtained by chemical analysis. This method has been controlled by chemical analysis in hundreds of experiments, and found perfectly accurate under the conditions stated above.

The time required for raising the concentration in the chamber *C* to its constant level is about thirty to sixty seconds. This was determined by vacuum samples taken at *L*. With a flow of 250 liters per minute the absorption by the skin and hair of the animal is reduced to a negligible quantity, the effect of any decomposition which may take place in the substance in air is reduced, and the time required to raise the concentration and clear the apparatus after the experiment, is short enough to be negligible in exposures over twenty or thirty minutes.

SUMMARY

An apparatus has been described, which is based on the apparatus used by Lehmann, for studying the effects of gases and vapor upon animals. The apparatus described is considered somewhat simpler and more convenient, as well as more generally applicable, than Lehmann's apparatus.

REFERENCES

- (1) LEHMANN: See Arch. f. Hyg. 1884, ii, 223; 1886, v, 11; 1887, vii, 231; 1893, xvii, 324; 1893, xviii, 180; 1898-99, xxxiv, 272, 308, 321, 335; 1904, xlix, 275 and 307; 1907, lx, 191; 1908, lxvii, 57 and 287; 1910, xlv, 1 and 307; 1911, lxxiv, 1; 1912, lxxvii, 311; 1913, lxxviii, 260 and 323.
- (2) VOIT: Zeit. f. Biol., xi, 532.
- (3) Lehmann's apparatus is described and pictured in Arch. f. Hyg., 1886, v, 11; 1898-99, xxxiv, 321; 1910, lxxiii, 307; 1913, lxxviii, 323.
- (4) LEHMANN: Arch. f. Hyg. 1898-99, xxxiv, 321.

ON DICHLORETHYLSULPHIDE (MUSTARD GAS)

III. SOLUBILITY AND HYDROLYSIS OF DICHLOR-ETHYLSULPHIDE. WITH A NEW METHOD FOR ESTIMATING SMALL AMOUNTS OF THE SAME

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INTRODUCTION

It became necessary to know in the use of aqueous solutions of dichlorethylsulphide in physiological experiments, the concentration actually obtained in a saturated solution as ordinarily prepared and the conditions under which such a solution should be handled to keep the rate of hydrolysis down to a point where the change in the concentration of mustard gas from this cause did not introduce an appreciable error into the experiment. A number of experiments were made.

SOLUBILITY AT 10°C.

Method. Water was cooled down to about 8 or 9°C. and an excess of dichlorethylsulphide was added to it in a separatory funnel. The funnel was then stoppered and shaken vigorously for several minutes and the temperature frequently observed to be sure that it did not rise above 10°C. When the mixture had nearly reached 10° the large globule which settles out at the bottom of the funnel was removed and at 10°C. the remainder of the solution was filtered rapidly into a bottle contained in a water bath at 10°C. in order to remove smaller globules. Care was used also to exclude the film of dichlorethylsulphide which is usually held on the surface of the mixture by the surface tension.

The temperature was frequently observed during the filtration and it did not vary more than 0.2°C .

Analysis of the saturated solution as obtained above, was carried out by two methods: (1) Hydrolysis by heating and titration of the HCl formed with $\text{N}/100$ NaOH ; (2) Hydrolysis and titration of the chlorides by the Volhard method, using an excess of $\text{N}/100$ AgNO_3 and titrating back with $\text{N}/100$ NaSCN . A large number of experiments showed that each of these methods was consistent and that they checked each other very closely when the technique of the end point of the Volhard method was mastered.

Results. In a series of four experiments the solubility at 10° was found to be 0.72, 0.75, 0.69, 0.70 mgm. per cubic centimeter. Hence it would seem that the solubility of dichlorethylsulphide in water at 10° is about 0.07 per cent.

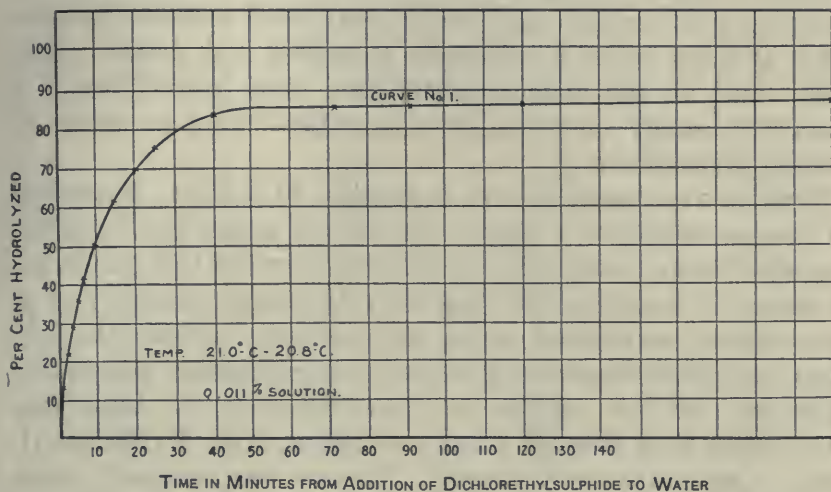
At higher temperatures, apparently higher concentrations than this are obtained because the hydrolysis is then rapid and as the dichlorethylsulphide in solution hydrolyzes, more will go into solution. At 10° , however, as will be seen from the curve for hydrolysis (curve 3) the amount of dichlorethylsulphide which hydrolyses in the time required to prepare the solution and separate it from the undissolved portion is slight. Allowing five minutes for the preparation of solution there would be according to the curve only 8 per cent of the total hydrolyzed.

HYDROLYSIS

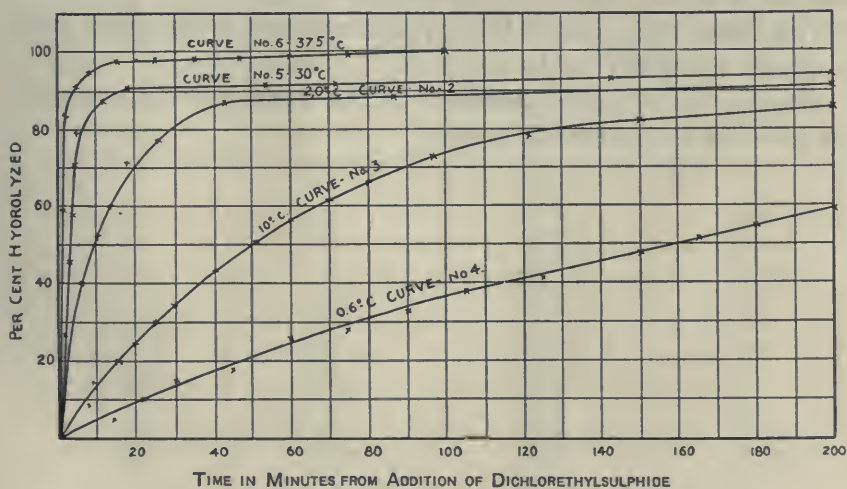
It is usually stated that the hydrolysis of dichlorethylsulphide in water is slow. As regards the hydrolysis of a mixture of dichlorethylsulphide and water when the former is in excess, the hydrolysis seems to be a slow process when the total amount of dichlorethylsulphide is considered. However, what we are dealing with are two processes, solution and hydrolysis. As the first dissolved portion hydrolyzes more of the dichlorethylsulphide goes into solution and this continues.

But when we consider the rate in a solution free from an excess the rate is rapid at ordinary temperature, as will be shown below:

HYDROLYSIS OF DICHLORETHYLSULPHIDE IN WATER



HYDROLYSIS OF DICHLORETHYLSULPHIDE



Method. A large bottle of about 2 liters' capacity containing 2 liters of water was placed in a water bath and the temperature adjusted to that desired; dichlorethylsulphide was added from a dropping bottle so as to give the approximated concentration desired, and the bottle immediately stoppered and shaken vigorously until all the dichlorethylsulphide went into solution. A minute is usually sufficient, providing there is not an excess of dichlorethylsulphide.

Portions were taken as soon as possible, 50 or 25 cc., depending on the concentration. These were allowed to run into small Erlenmeyer flasks contained in freezing mixture of CaCl_2 and ice, to arrest the reaction, the time for each being recorded just as the solution was allowed to run into the flask. At first the portions were taken about every minute as a preliminary experiment indicated that the reaction was very rapid at the beginning. These were then titrated when convenient, with $\text{N}/100\text{NaOH}$, using phenolphthalein (3 drops). No care was especially taken to use CO_2 free water, but as no appreciable blank was obtained on 50 cc. of the distilled water used, it is not likely that an error was introduced here. With a concentration slightly below saturation 25 cc. portions gave a convenient titration.

The temperature in all the experiments was maintained quite constant, especially in the one at 10°C .

Results. The values obtained in the hydrolysis experiments are given in the following tables:

Experiment I. Seven drops of dichlorethylsulphide were added to 2 liters of water, and the bottle stoppered and shaken. Temperature 21.0° to 20.8°C.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	2	0.80	13.23
2	3 $\frac{3}{4}$	1.31	21.68
3	5 $\frac{1}{2}$	1.79	29.62
4	6 $\frac{3}{4}$	2.25	37.23
5	8	2.50	41.36
6	9 $\frac{3}{4}$	2.75	45.50
7	11	3.01	49.80
8	16	3.71	61.39
9	21	4.21	69.66
10	26	4.52	74.78
11	41	4.98	82.39
12	56	5.00	82.73
13	121	5.25	86.88
14	136	5.35	88.53
15	21 $\frac{1}{2}$	5.40	89.35

The reaction in this case was followed as far as 94 per cent.

Experiment II. Forty-two drops of dichlorethylsulphide were added to 2 liters of H₂O 2.32 $\frac{1}{2}$ -2.33 p.m. and shaken; 50 cc. portions were titrated. Temperature 20.5°C.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	6	14.90	40.0
2	11	19.25	51.9
3	15	22.40	60.2
4	18 $\frac{1}{2}$	26.40	70.9
5	25 $\frac{1}{2}$	28.40	76.3
6	28	29.20	78.5
7	42	31.90	85.7
8	57	32.8	88.1
9	87	33.0	88.7
10	19 hrs.	34.8	92.99

Another portion heated to complete the hydrolysis gave a titration of 36.62 cc. or 98 per cent. Analysis by the Volhard method being taken as 100 per cent. In general the curve shows the same characters as the preceding.

Experiment III. In this experiment a saturated solution of dichloroethylsulphide at 10°C. was used and the temperature held at 10°C.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	12	2.70	14.71
2	15	3.79	20.64
3	20	4.67	25.43
4	25	5.52	30.06
5	30	6.45	35.14
6	40	7.80	42.47
7	51	9.25	50.37
8	60	10.30	56.10
9	70	11.29	61.55
10	80	12.17	66.45
11	97	13.32	72.54
12	121	14.25	77.87
13	150	15.00	81.70
14	211	15.67	85.51

After heating the last four portions on a water bath in stoppered Erlenmeyer's for thirty minutes, an average titration of 18.26 cc. was obtained, showing that the hydrolysis was not complete after a period of 211 minutes. Further, after two days a portion was titrated showing a titration of 17.53 cc. proving the hydrolysis was still not complete, although the solution had been at room temperature for some time.

Experiment IV. A saturated solution at 0.6°C. was used.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	15	0.60	5.77
2	22	1.05	10.09
3	30	1.59	15.29
4	45	1.98	19.04
5	60	2.69	25.87
6	75	3.01	28.95
7	90	3.49	33.56
8	105	4.10	39.43
9	125	4.30	41.35
10	150	4.99	47.98
11	165	5.41	52.02
12	180	5.73	55.11

Some of these portions, as in experiment III were heated in stoppered Erlenmeyers and the total amount obtained. After one titration they were returned to the water bath and heated for fifteen-minute periods until no further hydrolysis took place. Four samples showed an average titration of 10.50 cc.

Experiment V. Fifteen drops of dichlorethylsulphide were added to 1 liter of water at 30°C. and shaken one minute.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	1 $\frac{3}{4}$	2.71	27.1
2	3	4.64	46.4
3	4 $\frac{1}{4}$	5.84	58.4
4	6	7.07	70.7
5	8	7.94	79.4
6	13	8.69	86.6
7	18	9.16	91.6
8	29	9.12	91.2
9	32	9.07	90.7
10	53	9.17	91.7
11	83	9.61(?)	96.1(?)
12	113	9.62(?)	96.2(?)
13	113	9.25	92.5
14	173	9.43	94.3

Two portions heated on a water bath to complete the hydrolysis gave an average titration of 10.03 cc.

Experiment VI. Fifteen drops of dichlorethylsulphide were added to one liter of water at 37.5°C.

NUMBER	TIME IN MINUTES	TITRATION	PER CENT HYDROLYZED
		cc.	
1	2	6.04	58.9
2	3	8.48	82.9
3	5	9.34	91.3
4	7	9.71	94.9
5	10	9.84	96.2
6	15	9.93	97.0
7	25	9.97	97.4
8	35	10.02	97.9
9	47	10.04	98.1
10	60	10.22	99.9
11	105	10.23	100.0

Velocity constants. From curve 1 and curve 2, which are practically identical, it may be shown that the reaction is of the first order, as in the first case, the concentration is 0.011 per cent and in the second, 0.067 or nearly seven times as great. The per cent hydrolyzed in a given time, therefore, being independent of the concentration when the temperature is constant, hence, the reaction is monomolecular, the constants are calculated from the formula:

$$K = \frac{2.303}{T} [\log_{10} a - \log_{10} (a - x)]$$

or using per cents,

$$K = \frac{2.303}{T} [\log (100 - P) - \log (100 - P_1)]$$

The values of K were calculated from those portions of the curves where the errors of time on the one hand and the errors of titration on the other, were the least.

K 0.6°C.	K 10°C.	K 20°C.	K 30°C.	K 37½°C.
0.004761	0.01318	0.04816	0.2152	0.3445
0.004485	0.01385	0.04761	0.2123	0.2680
0.004416	0.01191	0.04784	0.2084	0.2620
0.004094	0.01316	0.04553	0.1557	0.2579
0.004255	0.01327	0.03951		

From the above experiments it is evident that mustard gas is soluble in water to the extent of 0.07 per cent, that in water solutions at ordinary temperatures where there is no excess of mustard gas, hydrolysis is rapid, varying markedly, however, with changes in temperature, and that the hydrolysis follows the equation of a reaction of the first order.

A NEW METHOD OF ANALYSIS OF SMALL AMOUNTS OF DICHLORETHYLSULPHIDE

The above experiments on the solubility and hydrolysis of dichlorethylsulphide in water, suggested that vapor and air mixtures could be passed through water and the amount of mustard gas estimated from the increase in the hydrogen ion concentra-

tion. In this manner very small amounts of acidity can be accurately determined in a short time. This allows of a small sample and a saving of time and apparatus. A determination including the taking of samples, requiring not over ten minutes.

Absorption was tried out at first by using higher concentrations. The sample was passed through water at 40 to 60°C. and the resulting acidity titrated with N/100 NaOH. These results checked with the Volhard method and the "loss in weight" method. One would conclude that if higher concentrations were absorbed, lower ones would, also.

The only special apparatus needed consists of Pyrex glass test tubes of uniform size about 6 inches by $\frac{5}{8}$ inch to contain the standards, and some large test tubes about 8 inches by $\frac{3}{4}$ inch fitted with petticoat bubblers for absorption tubes. These were also of Pyrex glass, as it was found necessary to have a quality of glass which is quite insoluble. Ordinary glass cannot be used.

Method. A series of standards was prepared from HCl solutions from N/100,000 representing 0.000795 mgm. dichlorethylsulphide per cubic centimeter to N/1,000,000 representing 0.0000795 mgm. per cubic centimeter. Methyl red solution was added to each standard. Of all indicators tried this was the only one which gave satisfactory results.

When ready to take a sample, the bubblers were carefully cleaned, thoroughly rinsed, and allowed to drain. Twenty cubic centimeters of CO₂-free distilled water was pipetted into each one. They were connected in series of two's, heated to about 35°C. in a water bath, and the sample absorbed. When the absorption was finished the bubblers were removed from the absorption tubes and the contents of each series were mixed by pouring from one tube to the other. Ten cubic centimeters were pipetted into a smaller tube, indicator added and comparison made as in hydrogen ion methods. As 40 cc. were used, the reading of the standard X40 divided by the number of liters in the sample taken gives the concentration in milligrams per liter.

Results

SIZE OF SAMPLE IN LITERS	CONCENTRATION BY ANALYSIS	CONCENTRATION BY "LOSS IN WEIGHT"	SIZE OF SAMPLE IN LITERS	CONCENTRATION BY ANALYSIS	CONCENTRATION BY "LOSS IN WEIGHT"
2	0.011	0.011	4	0.0077	0.0087
2	0.015	0.011	4	0.0077	0.0087
2	0.015	0.018	4	0.0077	0.0087
2	0.015	0.018	4	0.0077	0.0063
2	0.017	0.018	4	0.0066	0.0063
4	0.0092	0.0107	8	0.0038	0.0046
5	0.0115	0.0107	8	0.0038	0.0046
5	0.0098	0.0107	4	0.0036	0.0038
3	0.0103	0.0108	4	0.0041	0.0037
3	0.0103	0.0108	8	0.0023	0.0016
3	0.0109	0.0108	8	0.0013	0.0016
3	0.0109	0.0108	8	0.0023	0.0016
3	0.0103	0.0108	8	0.0023	0.0016
3	0.0093	0.0108	8	0.0018	0.0013
3	0.0103	0.0130	8	0.0018	0.0013
3	0.0103	0.0130	8	0.0018	0.0013
3	0.0109	0.0130	8	0.0018	0.0013
3	0.0113	0.0130			

Precautions. The two principal errors which arise are caused by (1) incomplete absorption, and (2) solubility of the glass. The first is easily overcome by warming the water to 35°C., as above, and placing two bubblers in series. The apparent absorption of mustard gas in water is increased by rise of temperature, as hydrolysis is then almost instantaneous, and the hydrochloric acid formed is far more soluble than mustard gas. As regards the second point, it is extremely necessary that all glassware used be quite insoluble, as even the small amount of alkali dissolving from ordinary glassware is enough to change the hydrogen ion concentration considerably. This may take place even in the cold as experiments showed. Pyrex glass showed up very satisfactory and was used in the above experiments. It is, however, somewhat soluble, and this makes it necessary to take readings as soon as the samples are taken.

Advantages. (1) Reagents and apparatus are very simple and inexpensive. (2) A small sample may be used, reducing the labor considerably, and also allowing of a greater number of

analyses during an experiment. (3) The time saved is one of the greatest advantages. A sample may be taken and determined within five minutes.

CONCLUSIONS

1. The solubility of dichlorethylsulphide in water was determined and at 10°C. was found to be approximately 0.07 per cent.

2. The velocity of the hydrolysis of dichlorethylsulphide was determined at 0.6°C., 10°C., 20°C., 30°C., 37.5°C., and the data graphically represented in the form of curves. The hydrolysis was found to follow the equation of a monomolecular reaction.

3. From the results obtained in the experiments on solubility and hydrolysis, a new method of analysis, depending upon the hydrogen ion concentration, was developed which could be used in the determination of very small amounts in vapor-air mixtures.

In conclusion I wish to thank Dr. E. K. Marshall, Jr., for valuable suggestions, under whose direction the work was done.

STUDIES IN THE ELIMINATION OF CERTAIN OF THE DIGITALIS BODIES FROM THE ANIMAL ORGANISM

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I. INTRODUCTION WITH DISCUSSION OF LITERATURE

Our knowledge of the elimination of the various digitalis principles from the animal organism is slight though the subject has been studied frequently. The lack of greater progress along this line is attributable to various causes among which is the want of suitable methods of recovering the active principles of this group from animal tissues and excreta in the form of pure solutions, and of estimating these principles quantitatively in impure solutions of tissue extracts.

Much confusion has resulted from applying the results obtained with one principle to others of the group, and from assuming that results obtained with one species of animal are applicable to others, and even to man, without determining the conditions that modify the actions of the substances in question. Even the mode of administration is of importance, for example, it is impossible to produce any perceptible effect in rats by oral doses of ouabain thousands of times larger relatively than would be fatal to the cat or dog by intravenous injection. Many of these modifying conditions have come to be so well recognized that one is prone to forget that they were not so well understood when much of the work still cited was done.

This condition has been further increased by the confusion in names of various digitalis bodies, for example, the word "digitalin" is commonly employed, but it is meaningless without a qualifying term. It has been used to mean digitoxin, true digitalin, or a mixture of the latter with digitonin, a saponin-like substance. So the word "strophanthin" is often used incorrectly for ouabain, as well as for amorphous strophanthins from different sources, of different degrees of activity and probably of different degrees of purity.¹ Much of the strophanthus of commerce was formerly of uncertain origin, and even careful investigators were frequently misled concerning the source of the strophanthus and strophanthin with which they worked,

¹ See New and Nonofficial Remedies, American Medical Association, 1918, p. 98, for a brief discussion of the important digitalis principles.

but it is now generally assumed that we can obtain the drug of known botanical source.

We are not convinced, however, that all commercial specimens of strophanthus—even those obtained from reputable dealers—are sold under their correct botanical names. One of us (1) has recently examined a specimen of strophanthus seed which had been purchased from a prominent drug miller who assured him that it was genuine *S. Kombe*. It yielded a fixed oil agreeing in amount and in physical properties with that obtained by Catillon (2) from a specimen of *S. hispidus*, the authenticity of which he believed to be beyond question. Another specimen of *S. Kombe* obtained from H. H. Rusby yielded an oil of quite different physical properties.²

The greater number of the experiments with amorphous strophanthin in the present research were carried out with the same specimens that were used by Hatcher and Bailey in 1909 and 1910. When possible we give the commercial source of the strophanthin with its supposed botanical origin, but we cannot vouch for the accuracy of the latter. We have recently (1918) examined these specimens by means of the biological test on cats and find that they have not undergone any appreciable change since they were purchased.

Crystalline ouabain was isolated from ouabaio wood about thirty years ago by Arnaud (3) who described it accurately and

² We have at no time concerned ourselves with the chemical investigation of the different digitalis principles, though Hatcher and Bailey stated, only parenthetically, on the basis of accepted investigations of others, that amorphous strophanthin is methyl ouabain (J. Am. Med. Assoc., 1910, lv, 1697). Brauns and Closson (J. Am. Pharm. Assoc., 1913, ii, 604) take pains to point out an assumed error on the part of those authors, supposing that they referred to the *Kombe* strophanthin alone. This is a pure assumption on their part, for it was stated distinctly in a previous paper (J. Am. Med. Assoc., 1909, lii, 5) that the strophanthin of Merck, and that of Boehringer and Sons were used, and it was then fairly well understood that Merck's strophanthin was made from *S. Kombe*, while Boehringer and Sons made theirs from *S. hispidus*. The American firm of Merck and Company was unable to tell us in January, 1916, whether the strophanthin obtained from them in 1908 by Hatcher and Bailey was made from *S. Kombe* or *S. hispidus*.

named it ouabain. He also established its identity with that of the principle obtained from *Strophanthus gratus* (then called *S. glabre* du Gabon), but some sixteen years later Thoms (4) rechristened it "crystalline strophanthin-g," thereby causing confusion.

In the present article we shall refer to this substance as ouabain, but in practically every instance it was called "strophanthin" or "crystalline strophanthin" by the various authors cited. Some authors who have used the amorphous strophanthin refer to it as "crystalline," and it is frequently impossible to determine whether the amorphous strophanthin used was from *S. hispidus* or *S. Kombe*, but this is probably of little importance, since they appear to be identical in behavior in the animal organism. There is a crystalline Kombe strophanthin but it is rarely employed in pure form.

The following references to the literature are intended to be suggestive, rather than comprehensive, and some of the papers are cited here because of the fact that they are often quoted in a way that renders them misleading, and we wish to point out the errors to which they lead. It is obviously impossible to escape all of the errors into which others have fallen, especially when attempting to explain statements that have proved misleading to others, but we trust that we can show the need of circumspection in accepting generalizations concerning the actions of drugs of this group.

It is not convenient to classify strictly researches dealing with the fate of the digitalis principles in the animal body, but for the most part these are concerned with their disappearance from the blood stream; their fixation or distribution in the tissues, especially in the heart and liver; their detection and estimation in the urine; their decomposition by acids, alkalies, the digestive ferments, and by bacteria; and their disappearance from the alimentary canal.

Koellicker, 1857, is said to have extracted antiarin from tissues and tested the extract biologically, at least Hedbom (5) states that he followed Koellicker's method and refers to Koellicker's paper on Upas Antiar (6) but we find no description of such

extraction or tests in that article, in which Koellicker stated that further work would be done.

Polailon and Carville (7) state that the active principle of inée (strophanthus, almost certainly gratus, which contains ouabain), is not eliminated by the urine, saliva, or intestine of the frog, but that it accumulates in the blood. Their conclusion with reference to excretion is probably valid, but they used massive doses and it is not remarkable that they should have detected very small amounts in the blood of the frog. They also isolated enough of the poison from the blood of a dog to cause toxic symptoms in a frog. It is hardly justifiable to conclude from this that it, "accumulates" in the blood. Their results are commonly cited but they have little practical value in determining the fate of the digitalis bodies.

Hedbom (5) following the method of Koellicker—so he states—tried to extract antiarin from the hearts of severely poisoned frogs and to detect the substance by means of tests on the hearts of other frogs. His extracts exerted a weak action but he was unable to induce typical systolic standstill. His tests of the urine were negative.

Heuser (8) took advantage of the fact that the toad is resistant to the action of the digitalis principles to study the distribution of strophanthin (source not stated, probably the amorphous, from *S. Kombe*, then official in the German Pharmacopoeia), in the body. He found the toad's serum incapable of completely destroying strophanthin treated with it, and he was able to induce systolic standstill in *R. temporaria* with 1 cc. of the serum obtained from a toad that had previously had a dose of 2 mgm. of strophanthin. Since 2 mgm. are sufficient to kill about one hundred and fifty frogs of the size used for the test, the experiment can hardly be said to prove whether any destruction occurs or not. He could detect strophanthin in the intestine of the toad, a result which may be accepted.

Cloetta and Fischer (9) state that when digitoxin is shaken with a suspension of ground heart tissues and the mixture allowed to stand, the lower portion contains a relatively larger amount of the digitoxin than the supernatant fluid; liver and brain tissues

similarly treated do not carry down the digitoxin in combination. The authors worked with a secret preparation, known as digalen, which is said by Kiliani (10) to be nothing but a high percentage of digitalein, ("Das Digalen wird nichts anderes als hochprozentiges Digitalein"), but they make the amazing statement that their results are doubtless applicable to digitoxin.

Since digalen, which Cloetta arbitrarily called "amorphous" or "soluble" digitoxin, bears little resemblance to true digitoxin, the conclusions of Cloetta and Fisher have very little value and their assumption that the results are applicable to digitoxin has served merely to introduce confusion. Their paper is frequently cited in the literature by those who are apparently unaware of the true nature of the substance used. Of course it is a pure assumption, and it is an unwarranted one, to say that the results obtained by those authors are applicable to any other digitalis principle. As a matter of fact, they were attempting to explain why digalen was more active by subcutaneous injection than by vein, itself a false assumption, which they might have discovered by the simple experiment of injecting suitable doses of the drug subcutaneously and intravenously into animals. This paper is mentioned here merely as an example of those through which confusion has been introduced into the literature by careless generalizations.

Yernaux (11) found that digitoxin (there termed Nativelle's digitaline), disappears rapidly from the blood following its intravenous injection into the rabbit. He was unable to detect the poison by means of the biologic test on the frog's heart in blood taken one minute after the injection of the digitoxin. He found the chemic test for digitoxin unsuitable for the examination of blood.

Yernaux observed that digitoxin dissolved in horse serum (which is said to be a convenient vehicle) is more toxic by subcutaneous injection than an equal amount dissolved in alcohol or dilute alcohol.

Werschinin (12) found that the use of serum or blood as a vehicle for digitoxin or ouabain (there called strophanthin-g) greatly increased its toxicity over that in Ringer's solution,

presumably because some constituent of the serum increases the rate of penetration of the poison into the heart. He also found that lecithin (13) increased the rate of penetration of the poison into the heart but did not account for the increased toxicity, hence there is some other constituent of the serum that is concerned in this action.

Schliomensun (14) prepared from the hearts of men and animals a group of substances (alcohol-soluble phosphatids), which appeared to be capable of binding digitoxin, while corresponding fractions from skeletal muscles and liver had no such binding power.

Oppenheimer (15) found that serum, used as a vehicle, increased the activity of ouabain (there called strophanthin crystalline) and antiarin, but that it distinctly interfered with the activity of digitoxin, gitalin, digitalin (?), saponin, and methyl violet during perfusion of hearts of *Rana temporaria*. He maintained that lecithin and cholesterin are not responsible for the serum effect. He held that the disappearance of digitalis bodies (as indicated by the inability to detect them) that have passed through the organism cannot be attributed to destruction or to fixation in the cells.

Hatcher (16) found that when ouabain (there called crystalline strophanthin) is injected subcutaneously into the rat the larger part of it is excreted into the intestine, and a smaller part in the urine. The total amount recovered from the urine and intestine, as shown by the biologic estimation on cats, was nearly equal in one case to the amount injected. The mode of elimination into the intestine was not determined at that time. He administered a massive dose of ouabain (50 mgm.) to a rat by the mouth and recovered about 75 per cent of it from the gastrointestinal tract about twenty-six hours later. He was unable to obtain evidence that the heart tissues of the rat fix ouabain, despite the well known fact that that animal is extremely tolerant toward many of the digitalis bodies.

Hatcher and Bailey (17) sought to determine whether the liver of the dog is capable of fixing or destroying ouabain (there called crystalline strophanthin). They found no difference

between the amount required to cause death when the poison was injected slowly into the splenic, and that required when the injection was made into the femoral, vein. They were seeking to determine whether fixation in the liver accounts for the slighter toxicity of the poison after oral administration, as compared with that after subcutaneous injection. As a matter of fact, it is probable that very little of the poison is fixed during a single passage through the liver of any animal, except the rat, among those commonly used in laboratory experiments.

Dale and Laidlaw (18) tested the urine of the cat for apocynamarin biologically after the daily administration of doses of 2 mgm. for eight days. The results of the tests of three specimens of urine were uniformly negative. They state that the question of its elimination unchanged or its destruction in the body remains open, but they regard the negative results just mentioned as favoring the view that it is destroyed in the body. Apparently they did not take into consideration the possibility of its elimination in the bile.

Focke (19) states that the digitalis bodies combine with the cells of the heart muscle and that their slow excretion from the blood is the principal cause of the cumulative action that is characteristic of this class of poisons. Focke's statement is frequently quoted, but we are not aware of any experimental evidence upon which his assertion rests.

Straub (20) states that it is impossible to recover digitalis bodies from the poisoned hearts of frogs. The hearts were successively brought to systolic standstill with a solution of ouabain (there called strophanthin) with an apparent loss of 0.0002 mgm. of ouabain for each heart so brought to standstill. He concludes that this does not constitute "storage" of the poison in the heart in the sense in which that term is commonly understood, though he admits that storage may be said to occur to the extent of 0.0002 mgm. for each heart brought to standstill in that way.

Issekutz (21) found that frogs' hearts took up 0.00075 mgm. each of digitoxin, or about three and half times as much by weight as of ouabain, as observed by Straub. He believes that the digitoxin is firmly bound in the heart.

Gruenwald (22) argues that storage does occur in the heart. He employed Merck's "pure digitalin," which consists of a mixture of true digitalin and digitonin, and A. D. Waller (23) states that, "*Digitalinum pulverisatum purum Germanicum*" gives a characteristic record on muscle that is quite indistinguishable from a saponin record. Obviously we are not justified in accepting the results obtained with this digitalin as being applicable to any true digitalis body. See also Straub's reply (24) to Gruenwald and an article by Weizsaecker (25).

Lhoták (26) found that an amount of digitoxin equal to ten times the fatal dose disappeared from the blood of the rabbit almost immediately after its intravenous injection. He employed the frog test in the examination of specimens of blood taken from the rabbit immediately after death, which followed promptly the intravenous injection of very large doses of digitoxin. He was able to detect the poison in the organs of the animal after the intravenous injection of amounts equal to more than ten times the minimal lethal dose. Animals in a pathological condition with impaired functions, and those narcotized with alcohol or hydrated chloral did not eliminate digitoxin from the blood so rapidly as the normal animal.

The action was slower, and larger amounts were required to cause death, when the poison was injected into the crural artery than when it was injected into the femoral vein. About 20 per cent of the amount injected into the crural artery could be detected in the muscles supplied by that artery but none could be recovered from the corresponding muscles of the opposite side. He concluded that digitoxin is certainly fixed in the heart and walls of the vessels. He found that ouabain disappeared more slowly than digitoxin from the blood.

Lhoták also employed cross circulation between two rabbits, with a brief interruption in the cross circulation immediately after the injection of digitoxin. In some cases the injection of amounts of digitoxin equal to several times the fatal dose for the first animal failed to cause death in the other. We have cited Lhoták's results and conclusions at some length because of their immediate bearing on our own investigations.

Hatcher (27) injected digitoxin into cats, dogs, and a rat, and ouabain into cats and rats, and estimated the amounts of the poison remaining in the blood at death, or after various intervals of time, by injecting the defibrinated blood into another animal of the same species except in the case of the rat, the blood of which was tested by injecting it into cats. He found that about one-fourth of the digitoxin injected intravenously into dogs was present in the blood at death after six minutes; the greater part of massive and moderately large doses of ouabain disappeared rapidly from the blood of the cat, about one-third of the total amount injected intravenously being present in the blood at the time of death, which occurred within about two minutes. Ouabain also disappears rapidly from the blood of the rat. Owing to an oversight it is stated in Hatcher's paper that digitoxin disappeared completely from the blood of a rat in one minute; it should have been in one hour and forty minutes; no attempt was made at that time to determine the rate of disappearance of the poison from the rat's blood more precisely.

Hatcher also injected ouabain into the femoral artery of the cat and observed that death was delayed beyond the time when it would have occurred had the same dose been injected into the femoral vein. He concluded that these digitalis bodies are distributed widely through the tissues of the body following intravenous injection, but a footnote was added to the paper while it was in press stating that the liver of the rat fixes ouabain promptly after its intravenous injection.

Hatcher also reported that when a dose of 100 mgm. of ouabain is administered to a rat by the mouth the greater part of it disappears during the passage of the alimentary tract, being apparently destroyed by the successive action of ferments or by bacteria, since very little appeared in the feces and no symptoms of poisoning occurred despite the enormous amount used.

Clark (28) employed Kombe strophanthin only, prepared by Burroughs, Wellcome and Company, in an investigation of the mechanism of tolerance of the rat and snake to glucosids of the digitalis group. He tested extracts of the tissues on the frog's heart but found no evidence that any of the tissues of the rat, frog,

snake, or rabbit are capable of destroying strophanthin; that the red blood corpuscles of the frog in the presence of plasma possess the power of absorbing small quantities of strophanthin, but the mammalian blood has no such power. Perfusion of the rat's heart with a solution containing 0.005 mgm. of strophanthin in each cubic centimeter of the fluid caused systolic standstill in one hour; perfusion with a solution containing a total of 0.01 mgm. caused systolic standstill in four hours.

Especial attention is called to the fact that Clark worked with Kombe strophanthin, almost certainly an authentic specimen, and it is fair to assume that he used the amorphous. Unfortunately, Clark falls into the error of generalizing, at least in so far as the title of his paper is concerned—"The Factors Determining Tolerance of Glucosides of the Digitalis Series"—but in his conclusions he is careful to refer in every case to strophanthin alone. Clark also found no evidence that the liver of the frog, rabbit, or rat has any power to absorb strophanthin during perfusion experiments. We have taken pains to investigate the capacity of the rat's liver to fix amorphous strophanthin in view of the remarkable power that it exhibits for fixing the nearly related ouabain.

Groeber (29) suggested that the actions of amorphous and crystalline strophanthins from Kombe strophanthus and the amorphous strophanthin from hispidus strophanthus might be due to strophanthidin resulting from the decomposition of the strophanthin in the organism. He based this view on the observation that the effects of strophanthidin are elicited almost immediately after its intravenous injection in the rabbit, whereas there is always an interval of some minutes following the injection of the smallest effective doses of strophanthin before the effects are induced. There is no necessary connection between these two observations, however, and Groeber fails to explain why he found strophanthin to be about three and a half times as active as strophanthidin. He states that the question is still an open one.

The greater number of the active principles of the digitalis group are glucosids and many attempts have been made to de-

termine to what extent they are decomposed by digestive ferments, acids, and alkalis under conditions simulating those found in the alimentary tract.

Deucher (30) digested true digitalin with hydrochloric acid, with hydrochloric acid and pepsin, and with pancreatin and determined the degree of decomposition by means of the frog test. He concluded that 0.2 per cent hydrochloric acid decomposes true digitalin, and that the addition of pepsin increases the rate of decomposition, but that pancreatin is without effect on the digitalin.

Krause (31) states that the injection of diastase at once after the administration of strophanthin (source not stated) was followed by recovery in a series of experiments in which amounts equal to five times the minimal lethal dose were given. He does not state the mode of administering the strophanthin, but the context suggests that it was injected subcutaneously. This observation has never been confirmed, so far as we know, but, on the contrary, we found that the intravenous injection of diastase was without perceptible effect on the course and degree of the intoxication when Merck's amorphous strophanthin (Kombe?) was injected intravenously at the same time. Krause's statement is important if correct, but we are unable to accept it.

Loewy (32) extended Deucher's observations and investigated the action of acid, alkali, and acid with pepsin on strophanthin (amorphous Kombe?), helleborein, and infusion of digitalis. Strophanthin was not decomposed; helleborein was decomposed only to a slight extent, or not at all; Loewy's statement is ambiguous, but the protocol of his experiment shows no decided action; acid decomposed the infusion of digitalis, the addition of pepsin to the acid having little effect on the rate of decomposition.

Loewy's paper is not convincing. He found that the infusion of digitalis lost nearly half of its activity when it was allowed to stand at room temperature for twenty-four hours, becoming practically inert within a period of seventy-two hours. This is entirely at variance with every day clinical experience and with our own experimental evidence. We (33) found the infusion of digitalis to be remarkably stable when kept for several weeks under a variety of conditions, and in one case (unpublished) we

found that a specimen of the infusion which had been allowed to remain in an incubator at 37°C. for nearly three years retained about half of its original activity. No one, of course, denies that acids destroy glucosids in time.

Hatcher (34) found that the digestion of ouabain (there called crystalline strophanthin) and tincture of strophanthus (Kombe or hispidus?) with pepsin and acid, and with pancréatin and alkali for two hours resulted in some loss of activity, but the apparent loss was within the limits of error of the experiment.

Worth Hale (35) employed the frog test to determine the degree to which various digitalis bodies are decomposed under conditions simulating those in the human alimentary tract. He found that hydrochloric acid decomposed digitoxin slowly, the addition of pepsin then being without influence on the rate of decomposition, while pancreatic digestion did not affect the digitoxin; French digitalin, (said to consist largely of true digitalin) digitalin, and infusion of digitalis, behaved essentially like digitoxin, strophanthin, (probably amorphous from Kombe strophanthus) was decomposed more rapidly.

Lhoták (36) administered digitalis to rabbits by the mouth in amounts up to thirty grams and sought to determine whether it could be detected in the gastro-intestinal tract, blood, liver, heart, urine, or feces. He detected it in the stomach occasionally (which is obviously to be expected), never in the small or large intestine, organs or excreta. He concluded that the rabbit destroys digitalis in the stomach and small intestine, and that this capacity for destroying it increases with habituation. His results are not convincing that the destruction occurs in the stomach and small intestine alone, since absorption followed by destruction elsewhere may occur, and we know that rabbits are capable of disposing of digitoxin rapidly after its intravenous injection (37).

Holste (38) treated infusion of digitalis and solutions of crystalline digitaline, digitoxin, ouabain, Boehringer's strophanthin (probably the amorphous, from hispidus) and helleborein with digestive ferments, diastase, and emulsin for periods varying up to twenty-four hours. He found that the activity of the infusion is destroyed readily, that of digitoxin and strophanthin

less readily, and he concluded that much of the uncertainty following the clinical use of digitalis results from the splitting of the active principles by digestive ferments.

This paper is one of those that serve to confuse, rather than clarify this subject. It appeared with the prestige of having come from Schmiedeberg's laboratory, but it is not convincing. An examination of the results actually obtained does not afford evidence that any uncertainty in the therapeutic action of these agents is due to splitting by digestive ferments. The destruction of ouabain and digitoxin was unimportant in amount, and those agents do not usually remain for periods of twenty-four hours in the stomach or duodenum, where absorption occurs.

Johannessohn (39) found ouabain (there called crystalline strophanthin-g) much more resistant than amorphous and crystalline Kombe strophanthins to hydrochloric acid and gastric juice. He also found hydrochloric acid and pepsin together less actively destructive of strophanthin than hydrochloric acid alone in similar concentration, his explanation being that pepsin bound a portion of the acid. Johannessohn's conclusions are more in accord with known facts than those of Holste just mentioned.

Certain salient facts mentioned in the literature just presented may be reviewed here because of their relation to the results of our own experiments. Their discussion will be reserved until we have detailed these experiments, in which we have sought to determine so far as feasible, the several stages in the elimination or destruction of ouabain, amorphous strophanthin, and digitoxin in the rat, cat and dog.³

There has been no satisfactory evidence afforded hitherto that any of the glucosids of this group are fixed in any of the tissues or organs of the body in amounts that permit their recovery and quantitative estimation.

There is no convincing evidence that any of the digestive juices or their ferments has any important destructive action on any

³ We regret that our inability to secure a supply of rabbits has interfered with our plan to continue our studies with this animal, begun several years ago, but its behavior toward these principles probably differs from that of the rat only in degree.

of the digitalis glucosids following their therapeutic administration by the mouth. But of course it is well known that dilute acids destroy all glucosids, including those of the digitalis group, in time.

Digitalis is by far the most important member of the group from a clinical point of view despite its well known disadvantages, but it varies in its content of the several active principles, hence we preferred to employ the active principles in the purest form available in our investigations, though this method has a disadvantage in that we do not know whether any of these principles exist in the leaf in the form in which they are isolated, and the active principles contained in the leaf may therefore behave somewhat differently. There is some evidence—not conclusive however—that the isolated digitalis principles are not identical with those existing in the leaf, but, on the other hand, there is a fairly close agreement between the actions of digitoxin and those of digitalis when they are administered orally, or intravenously in moderate doses, in which case the effects of saponin-like substances do not play an important rôle as they probably do in the almost instantaneous death that results from the intravenous injection of massive doses of extracts of digitalis.

It will be convenient to present the results of our studies under several headings, but the experiments cannot be recorded in strict chronological order because observations made in one series often necessitated the reinvestigation of problems in other series by newer methods.

II. ELIMINATION OF OUABAIN IN THE RAT

Ouabain was chosen for the first of the series of experiments because it is the only readily available pure crystalline principle of the digitalis group.⁴ A further advantage of ouabain lies in the fact that it is easily soluble in water and can be extracted conveniently from animal tissues, and the amounts so recovered can be estimated quantitatively by means of the biologic test on

⁴ All of our specimens of ouabain contain traces of insoluble impurity, but this is without significance in its experimental or clinical use.

the cat without the necessity of purifying the extracts beyond such simple methods as precipitating protein by heat and subsequent filtration.

The rat was selected for use in the first series of these experiments because its extraordinary tolerance toward the various digitalis bodies permits of the administration without harm of such amounts that the recovery of a fraction of that administered suffices for its quantitative estimation by means of the biologic test on cats.

The cat reacts fairly uniformly toward ouabain which is fatal in intravenous doses of 0.1 mgm. per kilogram of weight, and this holds true whether the poison be dissolved in salt solution, in defibrinated blood, in urine (if not in an excessive amount of the latter), in extracts of feces, or in extracts of tissues, but the latter must be injected slowly in most cases.

The method of extraction and estimation used with unessential modifications in the present investigation may be described briefly as follows: Blood is defibrinated and diluted, if necessary, so that it contains about one part of ouabain in 100,000 parts of solvent, it is then injected slowly and continuously into the femoral vein of the test animal until death occurs, the duration of the injection being approximately an hour or an hour and a half. Since 0.1 mgm. of ouabain is fatal to a kilogram of animal, the total amount of ouabain present in the blood can be calculated from the amount of blood required to kill the test animal. From one to three tests were made, depending upon the accuracy required in the experiment, and the amount of material available. Urine is tested in the same way as the diluted blood, being diluted with normal salt solution when there is reason to suppose that it contains more than one part of ouabain in 100,000 parts of urine. Tissues are ground with sand, or in a hashing machine, heated on a water bath with water or normal salt solution, cooled, filtered, and tested in the manner just described. Feces are collected in alcohol, ground in a mortar, the alcohol evaporated, and the residue extracted with water or normal salt solution on a water bath, cooled, filtered, and tested in the same way as the defibrinated blood.

When the total amount of ouabain present in the tissue, or other extract is insufficient to cause the death of one test animal we employ a method which we shall refer to briefly as the "combined ouabain" method of estimation. This consists in injecting all of the extract, or so much as may be deemed expedient, usually not more than 50 cc. for each kilogram of body weight, and completing the injection with a solution of ouabain of known concentration in normal saline. The difference between the amount of ouabain thus required in pure solution, and 0.1 mgm. for each kilogram of the body weight of the test animal is the amount contained in the injected extract. This "combined" method of estimating the amounts of ouabain present is also applicable to the estimation of any other digitalis body, the activity of which for the cat is known. If 10 per cent of the normal fatal dose of ouabain is required after the injection of an extract of any digitalis body it follows that the extract contained 90 per cent of the fatal dose of that body, from which the total amount present in the extract can be calculated.⁵

*Absorption and destruction of ouabain in the alimentary tract
of the rat*

Since ouabain is excreted partly in the urine following its subcutaneous injection, its absence in amounts that could be detected in the urine of the rat following the oral administration of amounts equal to hundreds of times that which suffices for its detection in pure solution seemed to indicate that no absorption occurred from the alimentary tract, but we have sought to determine this point with certainty, and whether it is destroyed in the alimentary tract; if so where, by what means, and the approximate rate of such destruction.

Ouabain is fixed in the liver of the rat and excreted promptly into the intestine following its intravenous injection, as will be shown subsequently, therefore, it seemed possible that the failure to detect the poison in the urine following the oral administra-

⁵ For a discussion of this "combined" method see the paper of Hatcher and Brody, *Am. J. Pharm.*, lxxxii, 360, 1910.

tion of massive doses might have been due to its fixation in the liver and excretion back into the intestine. We have, therefore, examined the liver, urine, and feces of rats after tying the common bile duct and administering massive oral doses of ouabain.

Absorption of ouabain after oral administration

A white rat, weighing 210 grams, was anesthetized, and the common bile duct was tied and cut.

The animal received 80 to 90 mgm. of ouabain in suspension, orally.

The urine was collected for a period of 22.5 hours, during which no stools were passed; the animal was then chloroformed, and the liver, the gastro-intestinal tract with contents, and the kidneys with the bladder were removed and extracted; the extract of the kidneys and bladder being added to the collected urine before testing. The amounts found were as follows:

Gastro-intestinal tract and contents	50.1 mgm.
Urine, kidneys and bladder	0.7 mgm.
Liver	0.0 mgm.
Total	50.8 mgm.

The protocol of this experiment shows that less than 1 per cent of the ouabain administered orally to the rat was present in the urine, and that the liver contained none, hence less than 1 per cent of the poison appeared to have been absorbed. The intestine with its contents contained about half of that administered, and we must suppose that nearly all of that which disappeared was destroyed, and, as we shall show subsequently that the liver and tissues do not destroy large amounts of it, we must suppose that the destruction occurred in the gastro-intestinal tract.

In another experiment, of which a condensed protocol is given, the bile duct was tied, after which a dose of about 100 mgm. was administered orally, and the feces, collected for five days, contained only about 2.3 mgm. of the poison.

Protocol of experiment (condensed). White rat, weight 127 grams

August 29. Common bile duct tied during ether anesthesia. 1.35 p.m. 125 mgm. ouabain in emulsion into stomach through blunt needle; slight loss; actual dose lay between 100 mgm. and 125 mgm.

August 31. 4.00 p.m. No feces passed; urine collected, called first portion, diluted to 50 cc.

September 3. 11.30 a.m. Urine collected, second portion, diluted to 100 cc., feces extracted, made up to 100 cc.

Summary of results of tests:

Urine, first portion, contained	0.93 mgm. ouabain
Urine, second portion; contained	0.00 mgm. ouabain
Extract of feces contained	2.33 mgm. ouabain
Total recovered	3.26 mgm. ouabain

In order to determine the seat, and approximate rate, of destruction of ouabain in the alimentary tract of the rat measured amounts were administered orally, and after varying intervals of time we determined the amount of the poison remaining in the stomach and intestine, or in certain parts of the alimentary tract, and in some cases the amounts present in the feces.

In one experiment 20 mgm. of ouabain were administered to a rat by the mouth; the animal was killed after two hours, and the entire amount of the poison administered was recovered from the stomach. A similar dose was administered orally to another rat which was chloroformed after twenty-four hours; the gastro-intestinal tract and feces together contained 13 mgm. indicating the destruction of about one-third of the total amount administered, absorption being negligible. Another rat ate about 40 mgm. of ouabain, which had been mixed with cracker dust, during a period of about two hours. The animal was killed four hours after having eaten the poison, at which time, the stomach contained none, the small intestine contained 1 mgm., and the large intestine contained 20 mgm., the results indicating the destruction of about half of that administered. Four milligrams of ouabain were recovered from the gastro-intestinal tract of a rat twenty-four hours after the oral administration of 10 mgm.

These experiments show that ouabain is destroyed at a variable rate, but on the whole fairly rapidly, in the rat's alimentary tract and we undertook to determine the immediate seat of this destruction.

Four rats, weighing 125, 133, 145, and 195 grams respectively, were given equal amounts of ouabain, the dose being 10 mgm. in

every case. The smallest rat received the poison directly into the stomach through a blunt hypodermic needle which was passed down the esophagus; this method prevents troublesome regurgitation such as occurs frequently when the poison is placed in the mouth or throat. The second rat was anesthetized and the ouabain injected directly into the duodenum in order to avoid the action of the gastric juice; the third was anesthetized and the poison injected directly into the ileum immediately above the ileo-cecal valve with the intention of having it pass into the large intestine without its traversing the stomach or small intestine. The fourth animal received the ouabain by subcutaneous injection.

The feces of all of these animals were collected separately in alcohol for a period of four days, extracted, and tested in the usual manner. The animal that received the poison directly into the stomach excreted 2.5 mgm. in the feces; that which received it directly into the ileum excreted 0.8 mgm., and the other two did not excrete any. It is not a coincidence that the least destruction occurred in the animal in which the poison traversed the entire gastro-intestinal tract, while all, or very nearly all, of the poison was destroyed in those cases where it traversed only a part of it. The animal that received the ouabain directly into the stomach suffered no ill effects whatsoever, peristalsis continued actively and feces were passed abundantly, hence the poison remained for a much shorter time in the intestinal canal in this case than in the others; two of which suffered severe depression from the anesthesia and the operation of opening the abdomen, and one from the relatively large dose of ouabain administered subcutaneously. Strictly analogous results followed in a similar series of experiments with the closely related amorphous strophanthin.

The results of the experiments just detailed point to the large intestine of the rat as the seat of active destruction of ouabain, and they suggest strongly that it may be the only part of the gastro-intestinal tract in which destruction of the poison takes place actively. We have tried to determine approximately the rate of this destruction by injecting weighed amounts of ouabain directly into the cecum, or mixing them with the contents and ground intestinal tissue, allowing them to remain in an incubator

at 38°C. for some hours, and determining the amount of ouabain remaining undestroyed, by means of the biologic test on the cat. The results of the experiments were far from uniform, and in some cases little destruction, if any, occurred; in others the destruction was less than we anticipated in the light of the results with the living animal.

We have no data upon which to base a positive statement by way of explanation of the discrepancy, but it is obvious that the processes that go on in the intestine of the living rat are quite different from those that take place in an excised and incubated intestine.

It is hardly necessary to say that we had previously convinced ourselves by experimental evidence that extracts of intestinal contents could be injected intravenously in the cat without inducing perceptible effects, and without affecting the course of poisoning by ouabain. The injection of an excessive amount of extract of the contents of the dog's large intestine caused depression with loss of appetite lasting a week or more in one case. The extracts of the intestinal contents contain some substance having a feeble hypnotic action, but this is not observed with the relatively small amount obtained from the rat (if it exists in that animal). It may be mentioned here in passing, that extracts of the rat's liver sometimes induce emesis in the cat when administered orally, and the uncooked liver of the cat appears to induce emesis, when eaten by another cat, more actively than an equal amount of raw meat.

It is evident that the extraordinary tolerance of the rat toward ouabain administered orally rests primarily on its want of absorption, for destruction does not then take place until the large intestine is reached. It is an interesting fact also that this animal resists almost completely the absorption of ouabain, which is readily soluble in water (as well as that of amorphous strophanthin, which is even more soluble), while it absorbs readily the practically insoluble digitoxin, after their oral administration. It is of further interest to observe that the cat, dog, rabbit, and even man, do not differ fundamentally from the rat in this respect, but only in degree, enormous oral doses of ouabain having been survived by man.

The elimination of ouabain after subcutaneous injection in the rat

This has been the subject of a paper by one of us (Hatcher) and it requires only a brief discussion here.

The rat is nearly one thousand times as resistant to ouabain as the cat, in proportion to its weight, when the poison is injected subcutaneously. The cause of this tolerance is evidently different from that seen after oral administration, for absorption from the subcutaneous tissues occurs fairly promptly, and an animal may die within a short time after the subcutaneous injection of a fatal dose.

The fatal intravenous dose of ouabain for the cat is 0.1 mgm. per kilogram of weight, regardless of the rate of injection within wide limits, but the minimal fatal dose for the rat depends largely on the rate at which the poison enters the circulation. The rapid intravenous injection of as little as 12 mgm. per kilogram of weight may be fatal almost immediately, but an amount roughly equal to 100 mgm. per kilogram may be injected subcutaneously without causing death, although practically all of this huge dose enters the circulation and is in turn excreted within a few hours.⁶

The protocol in brief of an experiment serves to show the approximate rate of absorption of ouabain from the subcutaneous tissues of the rat and its elimination into the intestine.

Protocol of experiment

A rat, weighing 202 grams, received 10 mgm. of ouabain subcutaneously; the animal was chloroformed after one hour and fifteen minutes, and the liver and the small intestine were removed and extracted separately, and the extracts tested in the usual manner.

The extract of the liver contained 2 mgm. of ouabain, that of the small intestine 4.24 mgm.

The result of this experiment shows that 6.24 mgm., or more than twice the fatal intravenous dose, had passed through the circulation in a period of one hour and fifteen minutes without causing death.

⁶ The average fatal dose of ouabain for the rat by intravenous injection is about 10 to 15 mgm. per kilogram of weight when a solution of one part in two hundred of normal saline is injected within a few seconds, but death is usually somewhat delayed after the smaller dose.

One may suppose that this is approximately the maximum rate of elimination of ouabain of which the organism is capable, and this view receives support from the results of repeated subcutaneous injections of large doses at intervals of two hours. In one such experiment a total amount equal to fifteen times the single fatal vein dose was administered to each of two rats within a period of four hours. One of the animals survived, the other died one hour and fifteen minutes after the last dose was injected. As a matter of fact we shall have occasion to show that the liver of the rat is sometimes capable of removing ouabain from the blood even more rapidly than these experiments indicate, but the rate at which an organ can fix and remove a poison from the blood is not always an index of the rate of elimination of the poison from those organs upon which its essential, or toxic, action is exerted. We are inclined to speak of this latter as the "essential" rate of elimination, in order to distinguish it from that which may take place immediately after the poison enters the circulation. It is evident that we are dealing with an important factor in persistence of action, but this phase of the question cannot be discussed here; it may be said, however, that animals do not recover completely even as rapidly as the rate of elimination after subcutaneous injection would lead one to suppose, and even where recovery does eventually follow such large and repeated subcutaneous doses, there are symptoms referable to the central nervous system which persist for many hours after the administration of the last dose. It is more than possible that these symptoms are caused by the fixation of minute portions of the poison and their retention in the central nervous system.

The protocol of an experiment showing the effects of repeated doses of ouabain injected subcutaneously follows:

Protocol. White rat, weight 40 grams

10.00 a.m. 60 mgm. ouabain per kilogram subcutaneously in N. S. 1-200.

10.10 a.m. Sick, tremulous, prostrated.

12.00 a.m. Depressed but recovering; 60 mgm. ouabain per kilogram as before; symptoms much like those after preceding dose.

2.00 p.m. 60 mgm. ouabain per kilogram as previously. The animal had partly recovered by the following morning, but was tremulous and hyperexcitable. Complete recovery finally took place.

We have frequently confirmed experimentally the observation of Hatcher (16) that approximately 15 per cent of the ouabain injected subcutaneously into the rat may be excreted in the urine, but the rate of elimination in this way is quite variable, and at times almost negligible.

It is significant that in some experiments Hatcher recovered from the urine and intestine of the rat nearly all of the ouabain that had been injected subcutaneously when toxic, but not fatal, doses had been used, whereas in several experiments we failed to recover amounts comparable to those injected. It is unnecessary to give the protocols of these, but in the light of our experiments that show that destruction takes place in the large intestine of the rat, but apparently not in the small, it seems probable that differences in the amounts destroyed account for our want of concordant results. Intestinal peristalsis in the rodent depends to a remarkable degree upon the food taken, hence it seems probable that in some cases the poison excreted into the small intestine remained there for a considerable period of time, and thus escaped destruction, or that it was recovered from the large intestine before destruction had progressed far, whereas in other instances the delay occurred in the large intestine permitting of more being destroyed.

Several experiments of this series were performed as class experiments, the tests being conducted by students under our supervision, and the data are not complete in every case, nor are the intervals recorded with precision in all of those, but we will tabulate the results obtained so far as we can. It is of especial interest that in one experiment the liver was found to contain as much as 2 mgm. of ouabain after the subcutaneous injection of 10 mgm. because we shall have occasion to show that in some cases the poison is passed on into the intestine from the liver very rapidly after the intravenous injection of much smaller doses. This affords convincing evidence that the liver may store the poison

when it passes gradually into the circulation, as it does after its subcutaneous injection.

Table 1 shows the dose of ouabain in milligrams; the interval in hours (or approximately) after the injection and before the

TABLE 1
Showing the excretion of ouabain in the rat

WEIGHT	DOSE	RECOVERED IN		DESTROYED	INTERVAL	
		Urine	Feces			
After oral administration						
<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>per cent</i>	<i>hours</i>
110	50.0		30.0	20.0	40	22
	50.0		37.5	12.5	25	26
	10.0		3.9	6.1	61	24
	20.0		13.6	6.4	32	24
150	40.0		21.0	19.0	48	4-6
125	10.0		2.7	7.3	73	96
165	100.0		25.0	75.0	75	48
(bile duct tied)						
127	100-125	0.93	2.33		97	50 118
210	80-90	0.70	50.1		40	22½
After intestinal administration						
145	10.0		0.9	9.1	91	96
135	10.0		0.0	10.0	100	96
143	10.0		8.5	1.5	15	22*
91	10.0		1.4	8.6	86	22*
After subcutaneous injection						
195	10.0	0.85	4.1	5.1	51	18
	15.0	3.00	10.0	2.0	13	24
	10.0		0.0			96
	10.0	0.16	2.8	7.0	70	24
270	15.0		4.6			24
297	5.0	0.60	1.1	3.3	66	18
180	10.0	1.80	2.9	5.3	53	18
200	20.0	3.00	4.8	12.2	61	48
85	8.5	0.50	2.5	5.5	65	24
110	10.0	1.50	4.9	3.6	36	24
150	10.0					96
150	10.0	12.0†		12.0	60	96

TABLE 1—Continued

WEIGHT	DOSE	RECOVERED IN		DESTROYED	INTERVAL	
		Urine	Feces			
After intravenous injection						
grams	mgm.	mgm.	mgm.	mgm.	per cent	hours
200	2.4	0.00	1.25	1.16	48	42
210	2.0	0.16	0.43	1.4	70	44
		(bile duct tied)				
	2.0†	2.12	0	0	0	20
230	2.0	1.15				44

* The intestine was tied above the cecum in both of these; the injection was made into the duodenum of the first, and into the cecum of the second.

† This amount was recovered from the urine and feces together of the two rats, each of which received a dose of 10 mgm.

‡ The bile duct was tied and this dose was administered ninety-six hours after a similar dose.

excreta were examined; the amounts found in the urine, and in the intestinal tract with its contents and any feces that were excreted during that period. In one experiment the intestinal tract was extracted after removing its contents but none of the poison was recovered from its tissues. In one experiment a rat, weighing 210 grams, excreted 0.17 mgm. of ouabain in its urine in forty-four hours after the intravenous injection of 2 mgm. The bile duct was then tied and the animal excreted 2.12 mgm. in the urine in twenty hours after a similar dose; this may possibly include a small residuum from the previous dose. Renal excretion after oral administration in the normal animal was disregarded in estimating the amounts destroyed.

The elimination of ouabain after intravenous injection in the rat

Ouabain leaves the blood stream rapidly after its intravenous injection, as previously stated, and we have undertaken to learn the mechanism of its removal and the paths that it pursues in the course of its elimination. We had previously found that the liver is concerned in the excretion of this poison, and earlier work had shown that a small part is excreted by the kidneys, after subcutaneous injection. We have endeavored, therefore, to deter-

mine whether all of the ouabain administered (excepting such minute amounts as must be taken up by the heart and central nervous system) is fixed at once by these organs.

Since nearly all of a subcutaneous dose of ouabain can be recovered from the urine and intestine of the rat within a few hours after its administration, and since its detection in the liver and blood of that animal presents no difficulty, we anticipated little trouble in recovering the poison from any of the tissues in which it might be fixed in notable amounts after the intravenous injection of doses that must be considered very large, in proportion to the fatal dose for the test animal, and in estimating the amounts so fixed. We were greatly surprised, however, to find that in some cases we were unable to recover nearly so much as we had injected when the tests were made shortly after the administration, whereas in other experiments larger relative amounts were recovered when the animals survived for longer periods.

This lack of concordant results caused us to repeat some of our experiments in order to determine whether the method of procedure was at fault, and to vary them for the purpose of attacking the problem in different ways. We will tabulate the results of the experiments of this series and discuss them in some detail. The table may give the impression that the experiments were haphazard, but most of them were performed for the purpose of solving definite minor problems, and space does not permit of our giving all of the protocols in full, but we shall give those which seem to us necessary for a clear understanding of the methods, as well as of the possibilities and limitations of these methods.⁷

⁷ Chemistry has so long been in the ascendancy that many refuse to accept the view that biologic tests may be preferable to the chemical in some cases. The following quotation serves to suggest the difficulties encountered in investigations of this character: "At present nothing definite is known regarding the fate of digitalis glucosids in the human organism, or the products into which they are changed or the forms in which they are eliminated. . . . Thus far it has not been possible to find any of the digitalis compounds mentioned above (digitonin, digitoxin, true digitalin) in blood or animal organs." (*The Detection of Poisons*, Autenrieth-Warren, 1915, p. 201.

Table 2 shows the dose of ouabain administered in milligrams; the duration of the injection and the interval, in minutes, following the injection before the death of the animal; the weight

TABLE 2

Showing the recovery of ouabain from the blood, liver, and intestine of the rat after intravenous injection

NUMBER	DURATION OF		WEIGHT	BLOOD		DOSE	OUABAIN RECOVERED IN		
	Injection	Interval		Total	Tested		Blood	Liver	Intestine
	min.	min.	gm.	cc.	cc.	mgm.	mgm.	mgm.	mgm.
1	15	5.0	156	9.8	4.5	2.0	0.25	0.49	
2	15	4.0	203	12.7	5.0	3.0	0.28	0.93	0.53
3	17	6.0	161	10.0	8.0	3.5	0.05	1.35	
4	10	2.0	73	4.5	2.25	1.5	0.06	0.52	
5	18	12.0	140	8.8	3.0	3.0	0.00	0.87	1.26
6	0	4.0	139	8.7	3.0	1.5	0.32		

Liver and small intestine extracted together in the following

7	9	5.0	166	10.4	5.0	2.0	0.05	1.13	
8	1	6.0	280	17.5	6.8	2.8	0.00	0.60	
9	1	6.0	185	11.6		1.8		1.10	

Perfused fluid and blood taken together in the following

10	8	1.5	202	12.7		3.0	1.33	0.80	
11	9	10.0	203	12.7		2.6	0.35	0.56	

The common bile duct had been tied in the following

12	9	31.0	123			2.0		1.04	
13	13	11.0	134			2.5		2.18	
14	10	11.0	230			2.0		0.35	
15	11	4.0	141			2.0		0.64	
16	16	154.0	150	9.2	9.2	3.0	0.00	0.97	
17	0	5.0	208	13.0	2.5	1.5	0.22		

The liver had been removed before injection in the following experiment

18	0	1.25	170	8.0	3.0	0.5	0.47		
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of the animal; the amount of blood used for estimating the ouabain remaining in the circulation; the estimated amount of blood in the body (calculated as one-sixteenth of the body weight in grams, and expressed in cubic centimeters of blood, the mean of

the figures given by Chisholm, taken from Donaldson's book on the rat); the calculated amount of ouabain remaining in the total blood of the body; the amount of ouabain recovered from the liver; and that from the intestine.

Discussion of experiments

Reference to table 2 shows the rapidity with which ouabain leaves the circulation and we may say with certainty that practically all of a non-fatal dose disappears from the blood within six minutes after its intravenous injection in the rat.

The condensed protocol of experiment 1 will serve to illustrate the method used in estimating the rate of disappearance of the poison from the blood; one of the tests involves the employment of the "combined ouabain" method to which reference has been made.

Protocol of experiment 1. White rat, weight 156 grams

9.50 a.m. 0.5 mgm. ouabain in normal saline 1-200 intravenously every five minutes; 4 doses in fifteen minutes.

10.10 a.m. Exsanguinated by incising heart, 4.5 cc. blood, blood defibrinated, diluted to 50 cc. with N.S. Liver, 5.2 grams, extracted to make 71 cc.

Tests

Diluted blood. Cat, weight 3.25 kgm.

10.53-12.20 p.m. Injected all of diluted rat's blood intravenously.

12.25-12.45 p.m. Injected 0.213 mgm. ouabain total; death.

Calculation.

Fatal dose of ouabain for cat of 3.25 kgm. is 0.325 mgm.

Ouabain injected in pure solution 0.213 mgm.

Difference due to ouabain in blood, 4.5 cc., is 0.112 mgm.

Total amount in blood of rat's body, 9.8 cc., is 0.25 mgm.

Extract of Liver; cat, weight, 2.7 kgm.

1.05-2.34 p.m. Injected 39 cc. extract of liver; convulsions, death.

Cat, weight 2.0 kgm.

3.04-3.59 p.m. Injected 28.5 cc. extract of liver; convulsions, death.

Calculation.

$39.0 \div 2.7$ is 14.4 cc. \times kgm.

$28.5 \div 2.0$ is 14.2 cc. \times kgm., average 14.3 cc. \times kgm.

Since 14.3 cc. equals 0.1 mgm. ouabain, 71 cc. equals 0.49 mgm.

In experiment 5 the liver and intestine contained more than 2 mgm. of ouabain at death, which occurred twelve minutes after completing the injection of a total of 3 mgm., and thirty minutes after beginning the injection. Only a small amount was recovered from the remainder of the tissues, and it is probable that this was present in the kidneys or urine. Not a trace of the poison could be detected in the blood; all of the blood that we could obtain by exsanguinating the rat was injected into a cat weighing 2.5 kgm., after which it required the full average fatal dose of ouabain.

In order to determine whether the intestine is capable of fixing ouabain and removing it from the blood, the following experiment was performed:

Protocol of experiment 13. White rat, weight, 134 grams

9.56 a.m. Common bile duct tied during ether anesthesia.

10.13-10.26 a.m. Injected 2.5 mgm. ouabain into jugular vein.

10.37 a.m. Chloroformed; liver, stomach, and intestine removed.

The liver was extracted with enough normal saline to make 100 cc.; the stomach and small intestine with enough to make 50 cc.; the remaining tissues, excepting the skin, feet, and tail, with enough to make 150 cc.

The several extracts were tested as follows: Three cats were used for testing the extract of the liver; one cat took the entire filtered extract of the stomach and intestine after which it required the full average fatal dose of ouabain to cause death; one cat took all of the available extract of the remaining tissues and a little more than half of the average fatal dose of ouabain to cause death.

The results of the tests may be summarized as follows:

The extract of the liver contained	2.18 mgm. ouabain
The extract of the stomach and intestine	0.00 mgm. ouabain
The extract of the remaining tissues	0.24 mgm. ouabain
Total recovered	2.42 mgm. ouabain

It is clear that the intestinal epithelium is incapable of excreting ouabain, except possibly in traces, and that the poison which is found in the lumen of the intestine after subcutaneous or intravenous administration is first fixed in the liver and then excreted in the bile.

We recovered 97 per cent of the total amount of ouabain injected in this experiment, more than 80 per cent being present in the liver, although the animal lived only eleven minutes after the completion of the injection and only twenty-four minutes after its beginning. The average fatal intravenous dose of ouabain injected at once is 1.5 mgm. for a rat of this size. The fact that this rat took an additional milligram in thirteen minutes indicates the rapidity with which the liver removes the poison from the blood. The result agrees fairly well with those observed in experiments in which repeated subcutaneous injections were made to determine the rate of elimination.

A glance at the tabulated results shows that the amount of ouabain found in the liver and intestine is much less in nearly every case than that which was injected, though we have every reason for believing that all of it had left the blood at the time the examination of these tissues was made. The question arises, therefore, whether this failure to recover a larger proportion of the amount administered was due to faulty methods of extraction, or to the fact that the poison was fixed, at least temporarily, in other tissues, which later give it up slowly to the blood to be fixed in turn by the liver and kidneys and gradually excreted by them.

We have been unable to answer this question with certainty but we believe that the evidence points to the fixation of the poison in the liver, and in the kidneys to a less extent, and in these organs alone, except for the negligible amounts which are fixed in the heart and central nervous system.

We know that much of the ouabain is fixed in the vessels of these organs in loose combination at first, and that it can be removed from them by simple perfusion with normal saline; we believe that it then passes into a more stable combination in the cells of the liver, and probably in those of the kidney. We know

that a part is present in the liver in such a condition that it is not removed by simple perfusion, but can be extracted by maceration of the ground liver tissue with water, and we believe that this portion has passed into the bile ducts; we were inclined at first to believe also that that portion which is firmly bound in the liver cells was not extracted by us.

We have no proof that the ouabain is at any time present in the liver in such a form that we are unable to extract it, or that that which we do extract after the removal of a part by perfusion is present in the bile ducts, but the evidence is conclusive regarding the removal of a part of it by simple perfusion, and of the presence in the liver of another part that resists removal by perfusion but is extracted easily; and certainly the rapidity with which larger percentages of the amount administered pass through the liver into the intestine point strongly to the fact that the liver takes nearly all of the poison out of the blood.

We have made numerous efforts to settle this question definitely, but without complete success. It seemed to us that if any of the ouabain were fixed in the liver cells in such a state that we failed to extract it, a longer period of extraction with fresh portions of solvent, including water and alcohol, might remove some of this residual portion, or that digestion of the liver tissues with pancreatic extract in alkaline solution might free it and permit of its estimation. We have been unable, however, to extract a trace of the ouabain from the liver residue with alcohol, or fresh portions of normal saline after extraction by the usual method, and digestion of the liver has equally failed to show the presence of more than that extracted in the usual manner.

In one of these experiments the bile duct of a rat weighing 177 grams was tied (in order to prevent loss of ouabain into the intestine) and 1.8 mgm. of ouabain were injected intravenously during a period of one minute; after six minutes the animal was exsanguinated, the liver, weighing 7.0 grams, was ground with sand and divided into two equal portions; one portion was extracted and tested in the usual manner; the other portion was digested with 25 mgm. of tested pancreatic extract with the addition of a

little sodium bicarbonate for nineteen hours, after which it was extracted and tested. The two tests of the extract of the digested portion by the "combined ouabain" method gave closely concordant results, showing the presence of 0.5 mgm. of ouabain in that portion of the liver, corresponding to the presence in the entire liver of a little more than half of the amount injected. The tests of the extract of the undigested portion were not quite so concordant, one of them indicating the presence of a little more than 0.5 mgm. of ouabain, the average for the two indicating about 0.4 mgm., corresponding to 0.8 mgm. in the entire liver. No significance can be attached to the difference in the amounts shown by the two extractions, especially, since neither indicated more than we have commonly found by the usual method of extraction.

In another experiment intended to duplicate this the test of the extract of the undigested portion indicated the presence of more ouabain than was shown by that of the digested portion. This seems to afford evidence that the liver does not hold any ouabain after extraction by the usual method.

It will be recalled that Lhoták (36) believes that digitoxin is fixed in the walls of the vessels and in the heart of the rabbit immediately after the intravenous injection of massive doses. We give the protocols of two experiments showing the behavior of ouabain immediately after the intravenous injection of a just fatal, or non-fatal, dose.

In the first of the two experiments about to be detailed (no. 10, of table 2) the blood and perfused fluid contained far more ouabain than was found in the blood alone in any of the other experiments, the amount shown being approximately equal to half of that injected. It is true that the interval following the completion of the injection was shorter than in any of the others, but little less than in experiment 4, in which practically all of the poison had left the blood. The duration of the injection—eight minutes—certainly sufficed for the removal of much the larger part of the poison from the blood, as may be seen by comparison with the tabulated results of experiments 6, 8, 9, and 17 in each of which the period between the beginning of the injec-

tion and the death of the animal was less than in the experiment under consideration.

The injection of the blood and perfused fluid in the test animals in experiment 10 was made somewhat too rapidly, and the two tests show an unusual difference, one indicating the presence of 1.13 mgm., the other 1.54, but whether we take the higher, the lower, or the average, as we have done, the amount of ouabain shown is far greater than that found in the blood in any of the other experiments. As just stated, the injection into the test animals was made somewhat too rapidly, and the effect of this is to give results correspondingly low, but the error is negligible in any event.

The discrepancy shown in the results of the two tests of the fluid perfused through the body in experiment 11 is of a different character, and such discrepancies are occasionally unavoidable. The total amount of the poison present in this fluid did not suffice for a single complete experiment without resorting to the 'combined' method, and since we had decided to make two tests of each of the fluids in this experiment the amount of perfused fluid available for each of the tests contained only a small part of the ouabain necessary to cause death, hence a correspondingly larger amount of the pure solution of ouabain was used, and a slight difference in the susceptibility of the two animals resulted in multiplying the error. There is no reason to doubt, however, that the fluid perfused through the lower part of the body contained a small amount of ouabain. It is barely possible that this was derived from the blood, since a comparison of the color of the perfused fluid with that of a dilution of a known amount of blood seemed to show the presence of more than 1 cc. of blood in the fluid. It seems more probable that the greater portion of this small amount of ouabain, if not all of it, was derived from the kidneys by perfusion of their vessels. The injection of 20 cc. of normal saline into the thoracic aorta had served to remove the greater part of the blood from the vessels of the liver and gastro-intestinal tract, hence the ouabain found in the fluid subsequently perfused through the liver could not possibly have been present in the very small amount of blood mixed with this

perfused fluid from the liver. We estimated that this perfused fluid contained 0.5 cc. of blood, or about 4 per cent of the entire amount in the rat's body.

Protocol of experiment 10 (condensed). White rat, weight 202 grams

9.52-10.00 a.m. 3 mgm. ouabain intravenously.

10.01-30 a.m. Heart stopped.

A cannula was introduced into the arch of the aorta, and normal saline perfused through the vessels, 91 cc. of bloody fluid being recovered; this was added to the blood, the mixture defibrinated, strained, and the whole made up to 150 cc.

The liver, weighing 11.5 grams, was excised and extracted, the extract measuring 61 cc.

The kidneys were extracted and the extract made up to 27 cc.

The brain and cord were extracted and the extract made up to 50 cc.

Tests

Blood (with perfused fluid).

1. Female cat, weight 3.04 kgm.

10.37-11.58 a.m. 9.7 cc. per kgm. injected intravenously; death.

2. Male cat, weight 3.25 kgm.

10.46 a.m.-12.23 p.m. 13.2 cc. per kilogram injected intravenously; death.

Calculation. First test — 9.7 cc. indicate 0.1 mgm. ouabain.

Second test—13.2 cc. indicate 0.1 mgm. ouabain.

If 9.7 cc. contain 0.1 mgm. then 150 cc. would contain 1.54 mgm.

If 13.2 cc. contain 0.1 mgm. then 150 cc. would contain 1.13 mgm.

Average

1.33 mgm.

Extract of liver:

Cat, weight 3.48 kgm.

1.15-2.37 p.m. 7.47 cc. extract per kilogram intravenously; death.

Calculation. 7.47 cc. indicate 0.1 mgm. ouabain; 61 cc. = 0.8 mgm.

Extract of kidney.

Cat, weight 2.16 kgm.

1.34-1.50 p.m. 7.4 cc. extract per kilogram.

1.50-2.11 p.m. 0.08 mgm. ouabain per kilogram; death.

Calculation.

7.4 cc. indicate (0.1-0.08) 0.02 mgm. ouabain.

27.0 cc. contain 0.07 mgm. ouabain.

Extract of brain and cord:

Cat, weight 3.18 kgm.

1.25–2.15 p.m. Injected all of the extract intravenously.

2.18–3.00 p.m. Injected 0.102 mgm. ouabain per kgm.; death.

Summary.

Blood and fluid	1.33 mgm. ouabain.
Extract of liver	0.80 mgm. ouabain.
Extract of kidneys	0.07 mgm. ouabain.
Extract of brain and cord	0.00 mgm. ouabain.
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Total	2.20 mgm. ouabain.

Protocol of experiment 11 (condensed). White rat, weight 203 grams

10.50–11.00 a.m. 2.6 mgm. ouabain intravenously; death at once.

Exsanguinated, obtained 5 cc. of blood, 20 cc. normal saline injected into thoracic aorta, the fluid collected and added to blood, which was defibrinated, strained and made up to 100 cc.

A cannula was placed in the portal vein and the liver perfused with normal saline, 100 cc. of fluid being obtained; this was of a bloody appearance and a comparison of the color with that of a dilution of a known quantity of blood indicated the presence of about 0.5 cc. of blood.

A cannula was introduced into the thoracic aorta and the lower part of the body perfused, 100 cc. of fluid being obtained, in which it was estimated there was more than 1 cc. of blood.

The liver was extracted in the usual manner, the extract measuring 200 cc.

Tests

Blood. 1. Cat, weight 2.32 kgm.

12.00–2.26 p.m. 23 cc. \times kgm. injected.

2.26–2.34 p.m. 0.016 ouabain \times kgm. injected; death.

2. Cat, weight 3.64 kgm.

12.14–2.27 p.m. 10.6 cc. \times kgm. injected.

2.27–3.52 p.m. 0.064 mgm. ouabain \times kgm.; death.

Perfused fluid of liver:

1. Cat weight 2.38 kgm.

12.29–2.09 p.m. 17.9 cc. perfused fluid \times kgm. injected.

2.30–3.40 p.m. 0.08 mgm. ouabain \times kgm. injected; death.

2. Cat, weight 2.58 kgm.

12.38-3.00 p.m. 21.5 cc. fluid \times kgm. injected.

2.30-3.59 p.m. 0.075 mgm. ouabain \times kgm. injected; death.

Perfused fluid from body.

1. Female cat, weight 2.64 kgm.

1.45-2.45 p.m. 18.9 cc. fluid injected.

2.45-3.31 p.m. 0.062 mgm. ouabain \times kgm.; death.

2. Male cat, weight 2.96 kgm.

1.50-2.49 p.m. 15.9 cc. fluid \times kgm. injected.

2.56-4.01 p.m. 0.08 mgm. ouabain \times kgm. injected; death.

Extract of liver.

1. Cat, weight 1.98 kgm.

2.32-4.16 p.m. 26.6 cc. \times kgm. injected.

4.19-4.27 p.m. 0.028 mgm. ouabain \times kgm. injected; death.

2. Cat, weight 3.28 kgm.

2.33-4.05 p.m. 12.9 cc. injected.

4.14-4.51 p.m. 0.077 mgm. ouabain \times kgm. injected; death.

Summary.

100 cc. blood and perfused fluid (average)	0.35 mgm. ouabain
100 cc. perfused fluid of liver (average)	0.11 mgm. ouabain
100 cc. perfused fluid of body (average)	0.16 mgm. ouabain
200 cc. extract of liver (average)	0.45 mgm. ouabain

Total	1.07 mgm. ouabain.
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The condensed protocols of experiments 3 and 4 are also given as they afford further evidence that ouabain is at first loosely bound in certain of the vessels and that it is not merely present dissolved in the blood. It is not feasible to make as many experiments as we would wish with a view to determining whether ouabain is present in various tissues, but since we know that the kidneys fix amounts of the poison varying up to about 20 per cent of that injected subcutaneously, we may be certain that after its intravenous injection at least a portion of those small amounts which are recovered from the body exclusive of the liver and intestine come from the kidneys. At any rate, we have failed to find evidences of the presence of more than traces of ouabain in the voluntary muscles of the rat, even though they still con-

tained some blood, following the intravenous injection of a dose of 3 mgm. in experiment 2; and the amount found in the tissues remaining after the removal of the liver, intestine, skin, feet and tail, was no greater than one might expect to find in the kidneys alone.

It is difficult to explain why the vessels of the liver bind ouabain loosely while the vessels of other regions do not, but we must suppose that if the vessels of other regions were capable of binding it loosely, perfusion of those vessels, which in their total extent far exceed those of the liver, would result in the removal of notable amounts of the poison. We have not succeeded in removing more than small amounts in this way, however, and must suppose that other vessels than those in the liver and kidneys do not have the capacity for binding more than traces of the poison. This capacity for binding the poison must be related to the excretory function of these organs. Even though the poison had passed into a second stage in the vessels of other regions whereby perfusion failed to remove it, we should expect to recover it from the muscles and other tissues by extraction, were it fixed in their vessels (cf. experiments on cats).

It is probable that small amounts of ouabain are momentarily bound loosely in the vessels of other regions and are again rapidly taken up by the blood as the liver reduces its concentration of the poison, and thus after a few minutes only traces remain in the blood and vessels of other regions except the kidney. The distribution of the poison between the blood and vessel walls seems analogous to that which we have observed during perfusion of strychnin through the liver of the dog (40). In those experiments we showed that the liver (or its vessels) bound the strychnin loosely and that it could be removed by subsequent perfusion with fresh portions of Locke's solution.

We know of no method of further investigating this phase of the problem, but the explanation offered seems probable in the light of subsequent experiments on the cat, for we believe that the behavior of ouabain differs quantitatively, rather than qualitatively, in these two species, and we have been forced to the conclusion that the vessels of various regions fix ouabain in the cat after its intravenous injection.

Protocol of experiment 3 (condensed). White rat, weight, 161 grams

9.44-10.01 a.m. 3.5 mgm. ouabain injected intravenously; animal was killed and exsanguinated at once.

5 cc. of blood were collected, after which 5 cc. of normal saline were perfused through the aorta, the escaping fluid collected and added to the blood which was defibrinated, diluted to 50 cc., and tested.

The perfusion of the vessels through the aorta was continued with repeated passage of the first portion, and the perfused fluid finally made up to 290 cc. A comparison of a dilution of the blood with the perfused fluid indicated the presence of about one-fourth as much blood in the perfused fluid as in the 5 cc. of diluted blood.

The liver, which was practically bloodless, was extracted in the usual manner, and the extract made up to 50 cc.

One test was made of the blood; two of the perfused fluid, which showed close agreement; and two of the extract of the liver, which were in exact agreement.

The results of the test show:

Blood (estimated at 80 per cent of the whole)	0.042 mgm. ouabain
Perfused fluid	0.137 mgm. ouabain
Extract of liver	1.35 mgm. ouabain
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Total amount recovered	1.529 mgm. ouabain

Protocol of experiment 4 (condensed). White rat, weight, 73 grams

9.50-10.00 a.m. 1.5 mgm. ouabain injected intravenously.

10.02 a.m. Death; exsanguinated.

Blood, measuring 2.25 cc. (estimated one-half of whole) diluted to 25 cc.

Vessels perfused with normal saline to make 97 cc.

Liver, nearly bloodless, extracted, extract made up to 50 cc.

Skin, feet, and tail removed and the remaining tissues, weighing 59 grams, extracted, the extract made up to 200 cc.

The results of the tests (all by the "combined ouabain" method) show:

Blood (1 test)	0.06 mgm. ouabain.
Perfused fluid (2 tests)	0.52 mgm. ouabain.
Extract of liver (1 test)	0.50 mgm. ouabain.
Extract of remaining tissues (including kidneys) (1 test)	0.30 mgm. ouabain.
<hr/>	
Total amount recovered	1.38 mgm. ouabain.

In the effort to learn definitely whether the liver plays such a preponderant rôle in the immediate removal of ouabain from the blood of the rat (its ultimate rôle in eliminating the poison not being just now under consideration), and whether other organs are capable of fixing it temporarily, at least, we performed three experiments which seem to us to throw some light on the question.

The first of these experiments was made on a rat weighing 170 grams; the liver was removed, a dose of ouabain equal to one-fourth of the average fatal dose for an animal of this size was injected intravenously, and the blood tested after death, which occurred after sixty-five seconds. The protocol of this experiment shows that little of the ouabain, if any, had been removed from the blood.

We are well aware that numerous objections may be urged against the results observed in this experiment. The removal of the liver of an animal can hardly fail to influence the behavior of almost any poison after its intravenous injection even though it were not one of those that the liver fixes actively, for such a profound change in the circulation as this operation involves could hardly fail to have some effect, to speak of no other possible changes that the operation might induce.

While we are not prepared to accept this one experiment as proving that the other organs and tissues are incapable of fixing ouabain, it is difficult to believe that if the vessels in other regions were normally capable of removing the poison from the blood they would have lost that power so nearly completely with the removal of the liver. We believe that this experiment, taken with others, affords corroborative evidence that the liver does play such a preponderant rôle as we have ascribed to it.

The second of these three experiments was designed to show whether excluding the poison from the liver by tying the portal vein exerted any marked influence on its toxicity. Half of the normally fatal dose was injected into the jugular vein of a rat in which the portal vein had been tied. Death resulted within a few minutes. This experiment has many of the objections pertaining to the one previously described.

The third experiment was free from the major objections

urged against the other two. It was designed to determine whether a much larger dose of ouabain than the average fatal could be injected into the portal vein without causing death. This animal survived the injection of an amount equal to twice the average fatal dose for a period of nineteen minutes, showing that the poison is less active by the portal, than by the jugular, vein. An amount equal to five times the average fatal dose by the jugular vein was survived for six minutes. This is in striking contrast to the rapidity of the action after the removal of the liver, and also in striking contrast to the amount required to cause death in the previous experiment, in which an even smaller amount would probably have killed the animal. The result of the injection into the portal vein of the rat is also in strong contrast to that seen in the dog, in which there is no perceptible difference in the amounts required to kill whether the injection be made into the femoral, or the portal, vein.

Protocol of experiment 18 (condensed). White rat, weight, 170 grams

10.05 a.m. Liver excised without hemorrhage.

10.25 a.m. 0.5 mgm. ouabain injected into the jugular vein; the respiration ceased in forty-five seconds; heart stopped twenty seconds after the respiration; the animal was exsanguinated.

2 cc. of blood collected from heart and vessels.

1 cc. of blood collected from abdominal region.

The blood was diluted to 50 cc. after defibrination.

Tests

Cat, weight 2.0 kgm.

10.57-11.18 a.m. Injected all of diluted blood intravenously.

11.23-11.57 a.m. Injected 0.025 mgm. ouabain in pure solution; death.

Calculation.

Cat of 2.0 kgm. requires to cause death	0.20 mgm. ouabain
Cat received in pure solution	0.025 mgm. ouabain
Balance in 3 cc. of blood of rat	0.175 mgm. ouabain
Total blood of rat ($170 \div 16$)	10.6 cc.
Loss in liver (not by hem.)	2.6 cc.
	<hr/> 8.0 cc.

If 3 cc. blood contained 0.175 mgm. ouabain, 8 cc. contained 0.47 mgm.

Protocol of experiment (condensed). White rat, weight 166 grams

Portal vein tied during ether anesthesia.

2.47 p.m. 1.0 mgm. ouabain injected into jugular vein (6 mgm. \times kgm.). Animal became very sick at once.

2.55 p.m. Heart stopped.

Protocol of experiment (condensed). White rat, weight 162 grams

2.01 p.m. Began the injection of ouabain into portal vein during ether anesthesia.

2.13 p.m. 4 mgm. (24.7 mgm. \times kgm.) have been injected; injection interrupted by struggling.

2.16 p.m. Injection resumed.

2.26 p.m. 10 mgm. (61.7 mgm. \times kgm.) have been injected.

2.30 p.m. Respiration ceased.

2.32 p.m. Heart stopped.

When the ouabain reaches the duodenum after having passed through the common bile duct its further course is similar to that which arrives at that point after oral administration, but when a small amount, such as may be administered intravenously without causing death, reaches the duodenum a part of it escapes destruction in the large intestine and appears in the feces, although the total amount destroyed in the gastro-intestinal tract after the oral administration of a massive dose may be from ten to nearly one hundred times as great.⁸

In one experiment a little more than 0.5 mgm. of ouabain escaped destruction and was eliminated in the urine and feces after the intravenous injection of a dose of 2 mgm, about 1.5 mgm. having been destroyed, presumably in the large intestine. A

⁸ We have called attention (loc. cit.) to a somewhat analogous condition with reference to the destruction of strychnin during putrefaction. Ranke (Arch. f. Anat. u. Phys., lxxix, 1, 1879) administered strychnin to dogs in doses of 100 mgm. and buried their bodies in order to permit of their putrefying during various periods of time. Ranke was unable to detect strychnin by the chemical test after 100 days, but he was able to obtain evidences of its presence by means of the biologic test on frogs even after 330 days. It is difficult to understand why traces of strychnin should resist decomposition for so long a time under conditions which had destroyed vastly larger amounts in less than one-third of the period.

rat received 2.4 mgm. intravenously in another experiment; the feces collected for forty-two hours contained 1.25 mgm. of the poison, or more than half of the total amount injected, which must have passed through the large intestine.

Protocol of experiment (condensed). White rat, weight 210 grams

1.18-1.25 p.m. 2 mgm. ouabain injected into jugular vein; urine collected for twenty hours (first portion); urine collected for next twenty-four hours (second portion); feces collected for forty-four hours.

The urine of the first twenty hours was diluted to 75 cc.

The urine of the next twenty-four hours was diluted to 50 cc.

The feces of forty-four hours extracted to make 50 cc.

Summary of results of tests.

Urine of twenty hours contained	0.173 mgm. ouabain
Urine of next twenty-four hours contained	0.000 mgm. ouabain
Extract of feces contained	0.425 mgm. ouabain
Total amount recovered	0.593 mgm. ouabain

Protocol of experiment (condensed). White rat, weight about 200 grams

3.15-3.25 p.m. 2.4 mgm. ouabain intravenously.

The urine was collected for forty-two hours; diluted to 100 cc.

The feces collected for forty-two hours; extracted to make 200 cc.

Tests

Urine. Cat, weight 1.78 kgm.

10.50-11.25 a.m. Injected all of the diluted rat's urine intravenously.

11.25-11.53 a.m. Injected 0.092 mgm. ouabain \times kgm.; death.

Calculation.

Cat weighing 1.78 kgm. fatal dose 0.178 mgm. ouabain

Cat received 0.164 mgm. ouabain in pure solution

Balance due to urine of rat 0.014 mgm. ouabain

(This is obviously within the limits of error).

Extract of feces. Cat, weight, 1.64 kgm.

11.18-12.15 p.m. Injected 15.7 cc. kgm. extract; death.

15.7 cc. contained 0.1 mgm., 200 cc. contained 1.25 mgm.

(A second test was made but discarded owing to error of technic.)

Table 3 shows the doses of ouabain administered; the amounts recovered in the urine and feces (together with the fecal matter in the intestine alone when the period was too short to permit of excretion in the feces); the amounts destroyed, expressed in milligrams, and in percentages of the dose administered; the interval

TABLE 3
Showing elimination of ouabain in urine of rat

WEIGHT	INTERVAL	DOSE	OUABAIN IN URINE	
			Mg.	Per cent of dose
After subcutaneous injection				
<i>grams</i>				
110	24 hours	10.0	1.50	15.0
	24 hours*	10.0	0.16	1.6
297	18 hours	5.0	0.60	12.0
180	18 hours	10.0	1.80	18.0
200	48 hours*	20.0	3.00	15.0
85	24 hours*	8.5	0.55	6.5
210	2 hours	10.0	0.13	1.3
After intravenous injection				
200	10.0 minutes	2.4	0.14	5.8
166	14.0 minutes	2.0	0.14	7.0
202	9.5 minutes	3.0	0.07	2.3
210	20.0 hours	2.0	0.17	8.5
After intravenous injection and tying common bile duct				
210	20.0 hours	2.0†	2.12	106.0
230	{ 20.0 hours	2.0	1.09	55.0
	{ 44.0 hours		1.15	58.0

* These intervals are approximate.

† The bile duct was tied and this dose administered ninety-six hours after a similar dose.

following the administration of the poison during which the urine and feces were collected.

We have found a distinctly smaller percentage of ouabain eliminated by the kidneys after intravenous, than after subcutaneous, injection. We can offer no satisfactory explanation of this,

and it is possible that a larger series of experiments might show less difference than our experiments indicate. The liver is capable of fixing small amounts of the poison more rapidly and energetically than the kidneys, but it is possible that when it has fixed a small amount its capacity for further fixation diminishes until a portion of the poison is eliminated or until it has passed into a later stage of fixation. This would permit of its taking up a larger percentage of an intravenous dose than of the greater amount that can be injected subcutaneously without causing death.

When the ouabain that has been fixed in the liver of the rat, following its intravenous injection, is prevented from passing into the duodenum by tying the common bile duct the poison is redissolved in the blood, carried to the kidneys and eliminated in the urine quantitatively or to a great extent, little being destroyed in the body apparently.

We say that little is destroyed "apparently" because we recognize the fact that it is impossible to determine with certainty by our present method whether very small amounts are destroyed, and it is quite possible that small amounts are destroyed in every case, and much larger amounts in some. It is also possible that small amounts, too small to be detected by us, may be excreted into the intestine in all cases and that under the abnormal conditions of an occluded bile duct this method of elimination may become important, and since destruction in the large intestine may then keep pace with excretion into its lumen we fail to detect it. We believe, however, that we have furnished conclusive evidence that the intestinal epithelium does not excrete notable amounts of the poison under normal conditions.

It is difficult to explain why we have recovered all of an intravenous dose of ouabain from the urine in one case where the bile duct had been tied, and have found only a little more than half of the dose in the urine in another similar experiment except upon some such hypothesis. With less delicate methods than those employed we might well have overlooked the relatively small amounts of ouabain that are normally excreted by the kidneys, just as we now fail to detect any that may be excreted by the

intestinal epithelium in the normal animal. If such small amounts are normally excreted in this way it is probable that with the prolonged sojourn of traces of the poison in the circulation the amounts excreted directly into the large intestine and destroyed there may constitute a large proportion of the total dose.

It may appear like a contradiction of our previous statement to speak of a prolonged sojourn of traces of ouabain in the blood, but it must be borne in mind that we do not claim to be able to detect ouabain in amounts much less than 0.1 mgm., and if as much as 0.001 mgm. is taken up from the liver and eliminated during the period of time required for the blood to make a complete circuit of the intestinal path, the amount eliminated in this way in a few hours is sufficient to account for the difference between the amount administered to the rat and that found in the urine after tying the common bile duct.

The blood is obviously incapable of redissolving more than traces of ouabain, if any, during the time that it is fixed in the cells of the liver and undergoing excretion into the bile ducts, and even when it has passed into the bile ducts the blood takes it up very slowly, so that there are only traces present in the circulation. Since the liver has a much greater capacity than the kidneys for fixing the poison, it follows that the greater part of even these traces must be taken up again by the liver, and only a much smaller part by the kidneys, and possibly by other tissues, and the poison continues to be fixed in the liver again and again in constantly diminishing total amounts until all of it is either excreted or destroyed.

The relative capacities of the liver and kidneys to fix ouabain vary in different individuals, and probably in the same individual at different times, as shown by the varying amounts found in the urine and intestine in different experiments, and consequently the rate of elimination of the poison in the urine after the bile duct has been tied varies widely in different individuals.

This may explain why after tying the common bile duct we found all of the poison in the urine within twenty hours after its injection in one case, and in another such experiment the elimina-

tion in the urine was still in progress after twenty-four hours, in which case the total amount eliminated by the kidneys in forty-four hours was little more than half of that injected intravenously. In this experiment the amount taken up from the liver (including that excreted into the bile ducts) and excreted by the kidneys must have been less than 0.0001 mgm. during the period required for a given red blood corpuscle to make the complete circuit of the liver and kidneys, supposing, of course, that elimination was uniform and continuous. Naturally, this does not refer to the portion of the ouabain fixed in the kidneys immediately after its injection.

Renal activity in the rat depends largely on the amount of food taken when this consists largely of cabbage, and the depression following the operation of tying the bile duct may have influenced the rate of elimination in this time.

It will be observed, however, that in the experiment, the protocol of which is given, the amount of ouabain indicated in the urine was 106 per cent of the total intravenous dose. This animal had received a similar dose ninety-six hours previously, and while it is presumable that all of the first dose had been eliminated, by the liver mainly, it is possible that a small residuum remained in the body. We are inclined to believe, however, that the urine actually contained a little less than the test indicated.

Protocol of experiment. White rat, weight 210 grams

The common bile duct tied and cut during ether anesthesia.

1.03-1.15 p.m. 2 mgm. ouabain injected into the jugular vein.

The urine, 5.2 cc., was collected during a period of twenty-three hours, diluted with normal saline, filtered, and tested on each of two cats in the usual way.

Tests

1. Cat, weight 2.78 kgm.

10.02-10.32 a.m. 3.6 cc. urine (diluted to 75 cc.) intravenously; death.

2. Cat, weight 2.72 kgm.

10.07-10.36 a.m. 3.42 cc. diluted urine as in preceding test; death.

3.51 cc. diluted urine (average) indicate 0.1 mgm. ouabain; 75 cc. contain 2.13 mgm.

Summary

The fatal intravenous dose of ouabain for the rat depends somewhat on the rate of injection, but with the rapid injection of a solution of one part in two hundred parts of normal saline the average is approximately equal to 12 mgm. per kilogram of weight.

Ouabain leaves the blood stream rapidly, and after a few minutes not more than a trace—too small to be detected by our method—is present in the blood of the rat.

Much the larger proportion of an intravenous dose is fixed in the liver at once, or very shortly after the injection, and a smaller part is fixed in the kidney.

It is loosely bound in the vessels of the liver at first (and probably in those of the kidney) and then it may be removed by simple perfusion with normal saline; it appears to pass from the vessels into a more stable combination presumably in the cells of the liver (and in those of the kidney), it passes fairly rapidly from the cells of the liver into the bile ducts and thence into the duodenum; it is extracted easily after it passes into the bile ducts.

After ouabain passes into the duodenum of the rat a variable amount is destroyed in the large intestine, but a part of even so little as may be injected intravenously without causing death, may traverse the intestine and appear in the feces, while equal percentages of many times larger amounts may be destroyed, after the oral administration, in similar periods of time.

When the bile duct is tied, preventing the ouabain from passing from the liver into the duodenum, it is reabsorbed from the liver into the blood, carried to the kidney and excreted quantitatively, or to a very great extent, in the urine.

We have some evidence that a part of the poison injected intravenously is destroyed in the body, probably in the liver.

III. THE ELIMINATION OF AMORPHOUS STROPHANTHIN IN THE RAT

Toxicity of amorphous strophanthin

We planned to conduct a series of experiments with amorphous strophanthin similar to those with ouabain, as we supposed the

toxicity of this substance for the rat to be a little less than that of ouabain by intravenous or subcutaneous injection. This is far from being the case⁹ as reference to the tabulated results of our tests on the rat will show.

The average fatal dose of amorphous strophanthin for the rat by subcutaneous injection is about 20 mgm. per kilo, or about a fifth of the amount of ouabain required; the fatal intravenous dose of amorphous strophanthin is approximately 8.5 mgm. per kilo, or about one-third less than that of ouabain.

TABLE 4
Showing the toxicity of amorphous strophanthin for the rat

WEIGHT	DOSE	EFFECT
Intravenous injection		
<i>grams</i>	<i>mgm. X kgm.</i>	
52	9.0 (B. & S.)	Death in 7 minutes
44	9.0 (B. & S.)	Death in 7 minutes
52	8.5 (B. & S.)	Recovered
179	8.3 (Merck's)	Death in 8 minutes
92	8.0 (B. & S.)	Recovery after severe depression
52	8.0 (B. & S.)	Recovery after severe depression
Subcutaneous injection		
150	40.0 (Merck's)	Death in 29 minutes; bile duct tied
130	23.0 (Merck's)	Death within 2 hours
150	20.0 (Merck's)	Recovery, very sick for 7 hours
127	15.6 (Merck's)	Recovery
130	15.4 (Merck's)	Recovery

The average weight of the rats available at the time this investigation was undertaken was about 100 grams, and the amount of strophanthin that can be administered intravenously at once

⁹ We used a specimen of Merck's amorphous strophanthin, purchased February 21, 1910, supposed to have been prepared from *S. Kombe*, and a specimen purchased April 21, 1908, labeled "Strophantin chem. pure" which was made by Boehringer and Sons, supposed to be from *S. hispidus*. The biological test 1918 showed that the activity of these specimens had not changed materially, if at all, since their use by Hatcher and Bailey in 1910, the average fatal intravenous dose for cats being 0.187 mgm. per kilo of weight.

to an animal of that size without causing death does not suffice for more than two tests on cats of the average size, weighing 2.5 kgm. It is obvious that we cannot extract small fractions of the intravenous dose of amorphous strophanthin distributed in the organs and tissues of rats of that weight and estimate such fractions by our method, hence we were compelled to modify our plans with reference to the study of its behavior after intravenous injection.

Absorption and destruction of amorphous strophanthin in the alimentary tract of the rat

Amorphous strophanthin, like ouabain, is devoid of toxicity when administered orally to the rat because only a small portion of the oral dose is absorbed, and because excretion keeps pace with absorption, even when delayed peristalsis results in the absorption of unusual amounts.

The behavior of amorphous strophanthin in the gastro-intestinal tract of the rat is closely similar to that of ouabain. Not only does it resist absorption almost completely, despite its solubility, but this lack of absorbability is probably explained in the same way as that of ouabain. At any rate, it is not due to destruction in the stomach and duodenum where absorption would be expected to occur. After a massive oral dose, equal to many times that fatal by intravenous injection, only a small part appears in the feces, in most cases, showing that in the absence of absorption of notable amounts the destruction must take place in the intestinal tract, and we shall see that this destruction actually occurs in the large intestine, as that of ouabain does, and presumably by the same mechanism. A further parallelism with ouabain exists, in that when intestinal peristalsis is interfered with by disturbances of the appetite, and the poison remains in the large intestine for a period longer than that normally required for its passage, the destruction is greater than in those cases where its sojourn in the large intestine is shorter.

We give the protocols of several experiments showing: The absence or destruction of more than negligible amounts of amor-

phous strophanthin in the stomach and duodenum during a period of time more than equal to that normally required for food to pass through this part of the gastro-intestinal tract; the approximate degree to which the poison is absorbed from the alimentary tract under different conditions; and the degree to which it is destroyed after its introduction directly into the duodenum and into the ileum immediately above the ileo-cecal valve.

Protocol of experiment. White rat, weight 115 grams

10.15 a.m. 20 mgm. amorphous strophanthin directly into stomach. No perceptible effect.

2.15 p.m. Animal killed with chloroform.

The stomach and small intestine were extracted in the usual manner, the extract made up to 500 cc. and tested on two cats.

The average of the results of the tests indicated the recovery of 18.7 mgm. of amorphous strophanthin.

The results of this experiment indicate that practically none of the amorphous strophanthin was destroyed during a period of four hours. Had the animal been feeding during this period the poison would have passed beyond the duodenum.

Protocol of experiment. White rat, weight 136 grams

10.20 a.m. 50 mgm. amorphous strophanthin (B. & S.) directly into stomach.

The urine, measuring 30 cc., was collected during a period of seventy-two hours and tested on a cat weighing 1.54 kilograms, which required the injection of all of the urine to cause death. This indicates the presence of 0.29 mgm. of the poison in the urine, or somewhat more than one-half of 1 per cent of the amount administered to the rat.

The feces of the rat were also collected for seventy-two hours, extracted in the usual manner, the extract made up to 100 cc., and tested on each of two cats with closely concordant results, which indicated the presence of 8.24 mgm. of amorphous strophanthin in the fecal extract.

The feces of the rat were then collected for forty-eight hours, extracted, and the extract tested with entirely negative results.

The results of this experiment indicate the destruction of about 85 per cent of the amount administered, and are comparable to those observed in similar experiments with ouabain.

The mere fact that the oral administration of such massive doses (equivalent to about 40 times the fatal vein dose) is not followed by symptoms of any sort indicates that absorption must be very slow or that destruction must take place actively in the organs or tissues of the body, since so little is eliminated in the urine.

Owing to the similarity of behavior of amorphous strophanthin and ouabain in the alimentary tract, thus far observed, we conducted an experiment in which measured amounts were placed in the duodenum of one rat and in the ileum just above the ileocecal valve of another, as in a similar experiment with ouabain. The urine and feces were collected, the feces extracted in the usual manner, and the urine and the fecal extract tested in the usual way.

About 70 per cent of that injected into the duodenum and practically all of that injected into the ileum were destroyed; the total amount excreted in the urine of both rats was about 2.5 per cent of that administered, or relatively five times as much as in the experiment in which the poison was placed in the stomach of a normal animal. The disturbance of appetite from the operation caused delayed intestinal peristalsis, permitting of a longer period of absorption in one of the rats, and a longer period during which it remained in the large intestine of the other, resulting in extensive destruction, as in a similar experiment with ouabain.

It may appear that the difference in the disturbance of appetite and the resulting delay of the poison in the intestine are insufficient to account for the great difference in the degree to which destruction occurred in the two animals, but in the first case the unusual delay occurred while the poison was in the small intestine, and peristalsis was more nearly normal while it was in the large, whereas in the second animal the delay occurred in the large intestine almost entirely.

The similarity of behavior of ouabain and amorphous strophanthin in experiments of this type, and the absence of extensive de-

struction of ouabain in the incubated large intestine to which it had been added, seemed to render such incubation experiments with amorphous strophanthin unnecessary.

Protocol of experiment. White rat, weight 172 grams

1.50 p.m. 15 mgm. amorphous strophanthin (Merck's) into duodenum during ether anesthesia.

4.15 p.m. Animal has eaten little; no feces passed; forty-three hours after operation animal had eaten abundantly and feces were abundant.

Feces of sixty-seven hours collected, extracted, extract made up to 100 cc. Three tests were made on cats, the results indicating the presence in the fecal extract of 4.4 mgm. of the amorphous strophanthin.

White rat, weight 160 grams

2.05 p.m. 15 mgm. amorphous strophanthin (Merck's) injected into ileum immediately above ileo-cecal valve during ether anesthesia. Animal did not eat at all for more than twenty-four hours, no feces passed in that period. Ate abundantly and excreted feces abundantly in forty-six hours; feces collected for sixty-seven hours.

Feces extracted, the extract tested on one cat, weighing 2.5 kgm., which received practically all of the fecal extract, and then required 0.101 mgm. of ouabain per kilogram to cause death, indicating that the extract contained none of the poison.

The mixed urines of these rats were tested on one cat, the results indicating the presence of 0.74 mgm. of amorphous strophanthin.

Elimination of amorphous strophanthin after subcutaneous and intravenous administration

In order to determine approximately the rate at which the rat eliminates amorphous strophanthin from the body we administered toxic, but not fatal, doses which were repeated as often as possible without causing death.

In these experiments we injected amounts equal to 60 per cent of the fatal dose intravenously at intervals of an hour. One animal succumbed to the fourth dose which was administered three hours after beginning the experiment, and one hour after a total

amount equal to 180 per cent of the single fatal vein dose had been given. Since this dose, equal to 60 per cent of the normally fatal, actually caused death, it follows that an amount equal to 40 per cent of the fatal dose remained in effect, hence we must suppose that the animal had disposed of the difference between 40 per cent and 180 per cent, or 140 per cent, in three hours, or the equivalent of one fatal dose in about two hours.

Two other animals survived the administration of four such doses, but they were evidently very near death. We have shown that amounts of ouabain approximately equal to the single fatal vein dose were fixed in the liver of the rat within half an hour after intravenous injection, and this slower elimination of amorphous strophanthin (as indicated by slower recovery) suggests that the poison is eliminated by a different path, or that there is a great difference in the capacity of the liver for fixing the poison and removing it from the blood stream.

Rapid elimination permits of the subcutaneous injection of a much larger dose of ouabain than can be administered intravenously without causing death, but slow elimination results in the accumulation in the blood of a fatal dose after the subcutaneous injection of amounts of amorphous strophanthin more nearly approximating the fatal intravenous dose.

While we have employed these repeated subcutaneous or intravenous injections of a given percentage of the fatal dose as a measure of what we have previously referred to as "essential elimination," the problem is not so simple in every case as it might appear from the frequency with which a given percentage of the fatal single dose may be injected over a short period of time, because the fatal effect of a single dose may be exerted on one structure, and that of repeated doses on a wholly different structure. This shows that the poison exerts a more prolonged action on some structures than on others, and it suggests that the poison is also retained more tenaciously by those organs in which its action persists. Nevertheless, the method does give valuable information regarding the initial rate, as opposed to the completeness, of elimination of many poisons.

The rate of elimination of the greater part of a dose of ouabain

or amorphous strophanthin, is shown in the experiments, but certain structures in the central nervous system of the rat show a persistence of action leading to the accumulation of toxic effects, and we must suppose that these structures retain minute amounts of ouabain and amorphous strophanthin more tenaciously than the heart. The reverse appears to be true of several members of the group with reference to the cat, for the action on the heart of that animal persists much longer than the action on the vomiting center. The center in the rat which shows this persistence of action is concerned with clonic convulsions, which often appear late; the rat usually dies from respiratory paralysis following the intravenous injection of a fatal dose of ouabain or amorphous strophanthin, and this is said to be true of the rabbit also, but we find that cats die from the cardiac effects invariably.

The protocol of an experiment shows that amorphous strophanthin leaves the blood of the rat promptly, not more than traces of the poison being present after the lapse of eight minutes following the intravenous injection of a dose of 1.5 mgm. in a period of twelve minutes. It is quite possible that the blood contained even less than the traces of poison indicated by the test, such amounts being within the limits of error:

Protocol of experiment. White rat, weight 179 grams

12.00-12.12 p.m. 1.5 mgm. amorphous strophanthin (Merck's) into jugular vein.

12.20 p.m. Death.

The rat was exsanguinated by cutting across the heart, after which about 20 cc. of normal saline were injected into the aorta, the escaping fluid was collected and added to the blood, the mixture defibrinated, and tested on a cat weighing 2.4 kilograms.

The cat received all of the defibrinated blood and perfused fluid, and then required 0.089 mgm. of ouabain per kilogram to cause death. All of the blood and perfused fluid, therefore, contained an amount of amorphous strophanthin equivalent in activity to (2.4×0.011) 0.0264 mgm. ouabain, or a total of 0.05 mgm. amorphous strophanthin.

We also examined the livers, small intestines, and kidneys with bladders of two small rats following the intravenous injection of

repeated doses of amorphous strophanthin. The protocol of this experiment shows that the small intestines contained a small amount of the poison, the bladders and kidneys half as much as the intestines, and that none could be detected in the livers.

Protocol of experiment. White rat, weight 85 grams

9.37 a.m. 0.425 mgm. amorphous strophanthin (B. & S.) into jugular vein; immediate depression and hyperexcitability.

10.37 a.m. 0.425 mgm. amorphous strophanthin as previously; effects as before.

11.47 a.m. 0.425 mgm. amorphous strophanthin as previously.

12.50 p.m. 0.425 mgm. amorphous strophanthin as previously.

1.30 p.m. Animal died.

White rat, weight 92 grams

9.51 a.m. 0.74 mgm. amorphous strophanthin (B. & S.) into jugular vein.

10.51 a.m. 0.46 mgm. amorphous strophanthin as previously.

11.52 a.m. 0.46 mgm. amorphous strophanthin as previously.

12.07 p.m. Animal died.

The livers of both rats, weighing together 8.9 grams, were extracted and the extract tested on one cat which received about 70 per cent of the total extract of both livers; the cat then required slightly more than 0.1 mgm. ouabain per kilogram to cause death, indicating that the extract contained no amorphous strophanthin.

The small intestines of both rats, weighing 6.7 grams, were extracted together, the extract made up to 100 cc. and tested on a cat weighing about 3 kilograms; the cat received about half of the total extract of the intestines and then required 0.064 mgm. ouabain per kilogram to cause death; the result indicating the presence of a total of 0.33 mgm. amorphous strophanthin in both rats' intestines.

The kidneys and bladders of both rats were extracted together, the extract made up to 50 cc. and tested on a cat weighing 1 kgm., which received 34 cc. of the extract, and then required 0.042 mgm. ouabain per kilogram to cause death, the test indicating the presence of a total of 0.16 mgm. amorphous strophanthin in the kidneys and bladders of both rats.

The failure to find evidences of the presence of amorphous strophanthin in the livers of these two rats after the intravenous injection of a total of 3.3 mgm. would seem to support the contention of Clark that the liver of the rat does not fix this substance, but its presence in the intestines points to its elimination by the liver. We therefore tied the bile duct of a rat and injected several doses of the poison intravenously at intervals of an hour. The animal died after the lapse of fifteen minutes following the third dose and a total amount equal to about twice the single fatal vein dose.

The protocol of this experiment indicated that the liver contained about one-third of the total amount injected, and while the blood was not examined, the interval elapsing after the injection before death sufficed for the disappearance of practically all of the poison from the circulation, hence there is no question that this poison was actually fixed in the liver and not merely present in the blood in that organ. Furthermore, none of the poison was found in the intestine, a fact that affords support to the view that that found in the intestines in the previous experiment had passed through the liver.

We believe that these experiments also offer an explanation of the greater toxicity of amorphous strophanthin, as compared with that of ouabain, for the rat.

The liver is not only less active in fixing this poison and excreting it, but it probably gives it up to the circulation more abundantly than it does ouabain. Certainly the animal in which the bile duct had been tied succumbed to a smaller total amount relative to its weight than any of the normal animals which received similar repeated doses at similar intervals of time.

Protocol of experiment. White rat, weight, 188 grams

The bile duct was tied during ether anesthesia.

9.45 a.m. 0.9 mgm. amorphous strophanthin into jugular vein extreme depression at once; rapid improvement.

11.00 a.m. 0.9 mgm. amorphous strophanthin as previously; symptoms as before.

12.00 m. 0.9 mgm. amorphous strophanthin as previously.

12.15 p.m. Death; total of 2.7 mgm., or 14.4 mgm. \times kgm.

The liver, weighing 9 grams, was extracted, the extract made up to 100 cc. and tested on a cat weighing 2.66 kgm. The cat received 23 cc. of the extract per kilogram, and then required 0.028 mgm. of ouabain per kilogram to cause death. The test indicated the presence of 0.58 mgm. of amorphous strophanthin in the extract of the liver.

The small intestine was extracted and the extract tested on a cat which received more than half of the total amount, after which it required slightly more than the average fatal dose of ouabain to cause death, indicating that the intestinal extract contained no amorphous strophanthin.

We did not observe the influence of tying the bile duct on the toxicity of repeated doses of ouabain, but this operation did not appear to influence the toxicity of single doses, for example, a rat, weighing 123 grams, received an intravenous injection of 2 mgm. of ouabain in a period of nine minutes without causing death during the time which it was designed to have it live (thirty-one minutes); the animal was then killed with chloroform.

The kidneys appear to be capable of eliminating rapidly that part of the ouabain which is taken up by the blood from the liver and which is not fixed in the liver again, as previously mentioned, whereas this reabsorption of strophanthin appears to subject the heart and respiratory center to its continued action to a greater extent than in the case of ouabain.

A rat, weighing 170 grams, was used in such an experiment. The common bile duct was tied during ether anesthesia, after which a dose of 2.6 mgm. amorphous strophanthin was injected subcutaneously; the urine was collected for a period of forty-six hours and tested on two cats, the results indicating the presence of 0.84 mgm. of amorphous strophanthin in the urine. Two rats, weighing 151 grams and 155 grams, respectively, were used as controls of this experiment. The first received a subcutaneous injection of 2.26 mgm., the other of 2.32 mgm., and the urines were collected separately for forty-six hours. The urine of the first rat contained 1.4 mgm., that of the second, 1.5 mgm. of the poison. The dose of amorphous strophanthin used in this experiment corresponds to 15 mgm. per kilogram of weight, and it is

interesting to observe that while the controls recovered promptly, the dose being only about 75 per cent of the average fatal by this mode of administration, the animal in which the bile duct had been tied was very sick throughout the entire period of the experiment, and took no food; another died during the night following a similar dose per kilogram after tying the bile duct. There can be little doubt, therefore, that tying the bile duct does increase the toxic effect of amorphous strophanthin for the rat.

It is to be observed that tying the bile duct does not result in increased elimination of amorphous strophanthin by the kidney as it does with ouabain, in fact, in the experiment just presented only about 33 per cent of the total dose of amorphous strophanthin administered was excreted in the urine by the rat with the bile duct tied, whereas the controls excreted about 65 per cent in that way.

A larger proportion of a subcutaneous dose of amorphous strophanthin than of an intravenous dose is eliminated by the kidneys, as is the case with ouabain. Two rats received a total of 6.8 mgm. of amorphous strophanthin intravenously during a period of three hours; they were chloroformed one hour after the last dose, the small intestines of both animals, examined together, contained 1.4 mgm.; the kidneys and bladders contained 0.32 mgm., or about 5 per cent of the total amount administered. It may be argued that the duration of the experiment was insufficient to permit of the elimination by the kidneys of as much of the poison as in the experiments following subcutaneous injection. It is possible that such an objection may be valid, but the rapidity with which poison leaves the blood following its intravenous injection, together with the rapidity of its passage into the intestine, as previously shown, lead to the belief that very little is fixed in the kidneys after the first few hours. It has been suggested already that absorption from the liver may take place, but the animal disposes of the greater part of a non-fatal dose very rapidly, and it is probable that the amounts which act directly on the heart and respiratory center are far less than those with which we deal in our tests.

In no instance have we been able to recover nearly so large a

proportion of the amorphous strophanthin injected as that of ouabain found shortly after its injection in several experiments. We are unable to explain this satisfactorily. It is possible that amorphous strophanthin is decomposed into the similarly acting, but weaker, strophanthidin, as suggested by Groeber (29) but we have no evidence bearing on this. We do believe, however, that our failure to recover a larger percentage of the amounts injected points to the decomposition of a part of it in the body.

Summary

The behavior of amorphous strophanthin and that of ouabain in the rat present many points of similarity. Both are absorbed very slowly and only to a slight extent from the gastro-intestinal tract, and the portions so absorbed are excreted in the urine in part, and in part by the liver presumably.

They resist the action of the digestive ferments in the stomach and duodenum, but much the larger part of an oral dose is destroyed in the large intestine.

Amorphous strophanthin is much the more toxic by intravenous than by subcutaneous injection in the rat; this difference is apparently due mainly to the slower fixation and excretion by the liver.

A large part of an intravenous dose of amorphous strophanthin is fixed in the liver and excreted into the intestine; the kidneys probably excrete a larger percentage of a subcutaneous, than of an intravenous, dose, and as much as 65 per cent of a subcutaneous dose may appear in the urine. It is possible that a larger number of experiments might show that there is less difference in this respect than we have found.

Tying the common bile duct increases the activity of amorphous strophanthin for the rat, probably because of the reabsorption into the circulation of a part of that which has been fixed in the liver, and which is then prevented from passing into the intestine.

We have been unable to determine with certainty whether any part of an intravenous dose of amorphous strophanthin is destroyed in the liver or other organs of the rat, but such destruction seems probable.

The close similarity in the behavior of ouabain and amorphous strophanthin in the body of the rat renders a more detailed consideration of our experiments with the latter drug unnecessary, because it would be mainly a repetition of what was said concerning the experiments with ouabain.

IV. THE ELIMINATION OF DIGITOXIN IN THE RAT

The recovery and quantitative estimation of digitoxin in extracts of animal tissues and excreta involve far greater difficulties than are involved in similar experiments with ouabain, and we have been unable to follow the course of digitoxin after its administration to rats with the same degree of precision as in the case of ouabain. However, we have investigated its absorption from the alimentary tract; its destruction in the body; its disappearance from the blood; and its excretion, to a limited extent.

The following method of extraction was adopted with some modifications in minor details, dependent upon the nature of the material under investigation and the degree of purification rendered necessary by the presence of fat or other matter in the extract. Blood was defibrinated, diluted with normal saline and used without further treatment; tissues and excreta were ground with sand, treated with dilute alcohol (about 60 per cent) on a water bath, for a few minutes, the extract evaporated on a water bath, the residue taken up in a little dilute alcohol, and this solution used for the biologic test; or the extract was shaken with chloroform, the chloroform distilled, the residue taken up in a little dilute alcohol and the solution tested on cats.¹⁰

Extracts of the gastro-intestinal tract usually contained a considerable amount of a soap-like substance which we were unable to separate completely from the digitoxin. The extraction of the liver usually presented little difficulty. The amount of 60 per cent alcohol used for dissolving the final residue varied greatly with the amount of material under examination and the anticipated yield of the poison; when it was planned to use only one

¹⁰ When a mixture of three volumes of water and one volume of alcohol is shaken with chloroform, the latter removes nearly all of the alcohol.

TABLE 5
Showing toxicity of digitoxin for the rat

WEIGHT	DOSE	RESULT
Intravenous		
grams	mgm. per kgm.	
83	60.0	Fatal at once
197	34.0	Fatal in 1 hour, 41 minutes
190	26.0	Not fatal in 1 hour (chloroformed)
192	26.0	Not fatal in 1 hour (chloroformed)
137	25.0	Fatal in about 3 hours
184	25.0	Fatal at once
165	24.0	Not fatal in 4 hours (chloroformed)
179	20.0	Fatal in about 2 hours, 30 minutes
235	17.0	Fatal in 2 hours, 7 minutes
180	15.0	Fatal in 1 hour, 7 minutes
47	15.0	Fatal in 2 hours, 5 minutes
55	10.0	Fatal in 2 hours, 25 minutes
48	10.0	Fatal in 2 hours, 36 minutes
213	10.0	Fatal in 2 hours, 2 minutes
39	7.5	Normal after 24 hours
55	5.0	Hyperexcitable after 24 hours
Intramuscular		
155	65.0*	Died during night
80	63.0	Fatal in 5 hours, 55 minutes
100	50.0	Fatal after 7 hours, before 22 hours
210	48.0	Survived
229	44.0	Survived, hyperexcitable next day
235	42.0	Survived for 5 hours, 30 minutes
Subcutaneous		
165	120.0†	Fatal after 6 hours, 30 minutes (during night)
87	115.0‡	Survived for 24 hours (chloroformed)
56	89.0	Fatal after 26 hours, before 40 hours
236	42.0	Apparent recovery, death after 7 days
125	40.0	Survived

* Two doses of 32.5 mgm. \times kgm. each, with interval of 1 hour.

† Fatal after more than 6 hours, 30 minutes following a second dose of 60 mgm. \times kgm. with an interval of 2 days.

‡ Two equal doses with interval of 2 hours, 13 minutes.

TABLE 5—Continued

WEIGHT	DOSE	RESULT
Oral		
<i>grams</i>	<i>mgm. per kgm.</i>	
75	133.0	Died during night
39	125.0	Died during night
41	100.0	Died during night
55	75.0	Fatal after 30 hours, before 47 hours
103	49.0	Fatal after 48 hours
106	47.0	Fatal on fifth day
127	40.0	Fatal after 4, before 6 days
143	35.0	Survived
145	34.5	Survived
145	34.5	Survived
150	33.3	Survived

cat as the test animal not more than 5 cc. of dilute alcohol was used. Our object was to avoid purification as far as possible, since the necessary manipulation nearly always involves loss of active principle, and, as previously stated, extracts of rat's tissue and excreta can be injected intravenously into cats without interfering with the quantitative estimation of the digitalis bodies held in solution.

The average fatal intravenous dose of digitoxin for the cat is 0.35 mgm. per kilogram of weight, but it shows greater variation than ouabain for different individuals. The action of the minimal lethal dose is elicited more slowly than is that of ouabain, consequently with continuous administration there is a longer interval after the fatal dose has been injected before the typical symptoms of a fatal effect are induced, and the excess over the amount actually required is greater than with the more rapidly acting ouabain, involving a correspondingly greater error in the estimation of the amounts present in the fluid to be tested. This error in the estimation of digitoxin can be lessened in some cases by employing the "combined ouabain" method previously mentioned.

With the slow and continuous injection of a suspension of digitoxin in normal saline the fatal dose for the cat is about 0.5 mgm.

per kilogram, when the injection is made at such a rate that about an hour and a half are required for the administration of that amount of the poison, but the fatal dose by the "combined ouabain" method is about 0.35 mgm. per kilogram of weight.

While we give the results in figures that suggest extraordinary precision, the error involved in this series of experiments is almost certainly greater than that in the corresponding experiments with ouabain, nevertheless, it is not of sufficient degree to vitiate the conclusions drawn.

The average fatal intravenous dose of digitoxin for the rat is approximately 8.5 mgm. per kilogram of weight, but some animals survived for several hours the intravenous administration of amounts equal to two or three times the average minimal fatal vein dose. The minimal fatal dose by the vein is about equal to that of amorphous strophanthin and is materially less than that of ouabain. The fatal oral dose of digitoxin for the rat is about equal to that by subcutaneous injection, being approximately equal to 45 mgm. per kilogram. We have been unable to determine the exact average fatal dose of this poison for the rat by the several modes of administration without employing a larger number of animals than we felt justified in using. The figures given in the table showing toxicity are taken from various experiments in some of which the animals were killed after an interval of time previously selected, and which serve at least to show that the doses used were not more rapidly fatal.

Absorption and destruction of digitoxin after oral administration

A comparison of the effects seen after oral administration with those following intravenous injection affords a rough measure of the absorption which occurs within a period of a few hours, the action of digitoxin being so persistent that it is relatively unimportant in this respect whether a given amount is absorbed in two hours or in twice that period. A much larger total amount, however, may be absorbed within a day, during which time elimination almost keeps pace with absorption, for the table shows that amounts equal to two or three times the fatal intravenous

dose may be injected subcutaneously without causing death, and we must suppose that practically all of a subcutaneous dose eventually passes into the circulation.

The figures given in the table indicate the extreme variability in the absorption of digitoxin from the gastro-intestinal tract of the rat. The absorption is almost certainly influenced by the rate at which the poison traverses the intestine, and when that portion which has been absorbed gives rise to nausea and loss of appetite, with resulting diminished peristalsis, the poison takes longer to pass the small intestine, permitting of increased absorption. This probably results in the establishment of a vicious circle, and it may explain the fact that some of our rats were more or less continuously sick for periods up to five days following the oral or subcutaneous injection of a single dose of digitoxin, in which case the poison was probably slowly absorbed and excreted to a large extent.

We have attempted to determine approximately the percentage of the oral dose which escapes destruction and appears in the feces. In one experiment of this series practically all of the digitoxin administered was recovered in the feces; in another experiment the amount of the poison recovered was roughly one half of that administered; in two others we were able to recover only an insignificant fraction of the oral dose.

There is no reason to doubt that in some experiments very little of the poison was destroyed and that in others very little escaped destruction, but the results of these experiments give no clear indication concerning the seat of destruction. It is possible that a part of the absorbed poison is destroyed in the liver.

Elimination of digitoxin after intravenous and subcutaneous injection

Lhoták reported, as previously stated, that digitoxin leaves the blood of the rabbit almost immediately, but we found evidence of its presence in the blood of two rabbits one of which lived forty-nine minutes after an intravenous injection, the other having been killed by a medulla stroke ten minutes after the intra-

venous injection of an amount that was not much more than the average minimal fatal dose.

We have also examined the blood of rats after various intervals of time following the intravenous injection of digitoxin. None of the poison could be detected in the blood of two rats which were killed one hour after the intravenous injection of 5 mgm. into each. Not a trace of digitoxin could be detected in the blood of a rat which was exsanguinated five minutes after the intravenous injection of 4.15 mgm. The protocol of another experiment shows that nearly all of an intravenous dose of digitoxin had left the blood stream in five minutes, at which time the liver had certainly fixed a small amount.

Protocol of experiment. White rat, weight, 228 grams

12.07-12.08 p.m. 5 mgm. digitoxin (Merck's) 1-200 dilute alcohol and N.S. into jugular vein.

12.13 p.m. Exsanguinated, 10 cc. blood obtained, a little normal saline used to wash vessels.

White rat, weight 189 grams

12.30-12.31 p.m. 5 mgm. digitoxin injected as in preceding.

12.36 p.m. Exsanguinated as in preceding, 5 cc. blood obtained.

The mixed blood of both rats was defibrinated and diluted with enough normal saline to make 50 cc.

The livers of both rats were extracted, and the extract made up to 5 cc. with dilute alcohol.

Tests

Diluted blood.

Male white cat, weight 2.48 kgm.

2.10-3.35 p.m. Injected all of the diluted blood; began injection of ouabain 1-100,000.

4.12 p.m. Convulsions; total of 0.066 mgm. ouabain injected.

Since 0.066 mgm. ouabain is fatal to 0.66 kgm. of cat, the digitoxin in the diluted blood corresponds to the fatal dose for $(2.48 - 0.66 =) 1.82$ kgm., or $(1.82 \times 0.35) 0.63$ mgm. in 15 cc. of blood. According to the tables previously cited, the total amount of blood in these two rats would correspond to $(228 + 189 = 417)$. $417 \div 16 = 26$ cc., which would contain 1 mgm. of digitoxin.

Extract of liver.

Male cat, weight 2.86 kgm.

1 cc. of the extract was injected intravenously every twelve minutes until 4 cc. had been injected, and twelve minutes later the remainder of the available extract—0.7 cc.—was injected. Five minutes later the injection of ouabain 1-100,000 was begun and after twenty-nine minutes convulsions and death resulted, when a total of 0.1 mgm. had been injected. Since this amount of ouabain is fatal to 1 kgm. of cat, the extract of the livers must have contained enough digitoxin to kill 1.86 kilograms, indicating the presence of 0.65 mgm. in 5 cc. of the extract.

The urines of rats were examined after various intervals following the administration of digitoxin by the mouth, subcutaneously, and intraperitoneally, but no evidence of its excretion by the kidney could be obtained in the normal animal. Three rats received a total of 20 mgm. of digitoxin (15 mgm. to two intramuscularly, 5 mgm. to one intraperitoneally) the urines were collected for periods varying from seven to twenty-four hours, evaporated, the residue extracted with dilute alcohol and the extract tested on frogs with entirely negative results.¹¹

Traces of digitoxin were indicated by the test of the urine of a rat collected for four days following the subcutaneous injection of a total of 10 mgm. after the bile duct had been tied. The amount indicated—about 0.25 mgm.—lay within the limits of error, and it is probable that not even a trace of digitoxin was present in the urine.

The feces of this animal were collected during a period of six days, extracted, and the extract tested in the usual manner, though it seemed improbable that the poison could be excreted in the feces after the bile duct had been tied. The results of this experiment, the protocol of which is given, leave little doubt that nearly all of the digitoxin was destroyed in the body of the rat, and the fact that the animal recovered almost as soon as the normal animal following similar doses, despite the fact that its bile duct was tied, points to the liver as the probable seat of destruction.

¹¹ The urine of one rat, which received a total of 10 mgm., was collected for twenty-four hours, that of one, which received 5 mgm. intramuscularly, for about seven hours, the data concerning the collection of urine from the one that received 5 mgm. intraperitoneally are lacking.

Protocol of experiment. White rat, weight 236 grams

The bile duct was tied during ether anesthesia.

2.00 p.m. 5 mgm. digitoxin by vein, in 1-200 alcohol and normal saline.

2.15 p.m. 5 mgm. digitoxin by vein as previously.

The animal ate little during the next three days; on the fourth day after injection it had eaten fairly well.

Death occurred after seven, and before the end of nine days, the animal having eaten well and passed abundant feces before death.

The urine was collected for ninety-six hours, diluted to 25 cc. and tested in the usual manner; the feces were collected up to the time of death, extracted as usual, the extract made up to 15 cc. and tested.

*Tests**Urine.*

Female cat, weight 2.26 kgm.

2.52-3.00 p.m. Injected all of urine intravenously.

3.10 p.m. Began injection of ouabain, 1-100,000.

3.47 p.m. Convulsions, death; total of 0.151 mgm. ouabain.

Calculation.

Cat required equivalent of 0.226 mgm. ouabain to kill

Cat received 0.151 mgm. ouabain

Difference 0.075 mgm. ouabain

0.075 mgm. ouabain, equivalent of (0.075×3.5) 0.26 mgm. digitoxin.

Fecal extract.

Female cat, weight 2.2 kgm.

1.53-2.38 p.m. Injected total of 8.8 cc. extract.

2.56 p.m. Began injection of ouabain, 1-100,000.

3.23 p.m. Convulsions, death; total of 0.114 mgm. ouabain.

The calculated digitoxin content of the total fecal extract was 0.63 mgm.

Numerous efforts were made to determine whether digitoxin is fixed in the liver and excreted in the bile after the intravenous or subcutaneous injection of the poison, but we have been unable to recover more than a small portion of the amount injected in any case. Absorption from the subcutaneous tissue takes place slowly and elimination almost keeps pace with absorption unless a mas-

TABLE 6

Showing recovery of digitoxin from body or feces

WEIGHT	DOSE	INTERVAL	RECOVERED	WHERE FOUND
After intravenous administration				
<i>grams</i>	<i>mgm.</i>		<i>mgm.</i>	
165	4.0	4 hours, 5 minutes	1.5(?)	Intestine
197	6.7	1 hour, 40 minutes	1.4	Intestine
192}	5.0	1 hour	} 0.0	Liver, small intestine
190}	5.0	1 hour		
137}	3.4	* { 3 hours 2 hours, 30 minutes 2 hours	} 1.0	Liver, small intestine
179}	3.6			
189}	2.7			
After intramuscular or subcutaneous injection				
87	10.0†	24 hours	0.6	Intestine and feces
75	10.0‡	20 hours, 30 minutes	Traces	Intestine and feces
200	10.0§	2 days	Traces	Intestine and feces
56	5.0	26-40 hours	0.0	Intestine and feces
125	5.0	5 days	0.8(?)	Feces
80	5.0	6 hours	0.0	Intestine
210	10.0	23 hours	1.6	Intestine and feces
235	10.0	5 hours, 30 minutes	0.8	Intestine
100	5.0	7 hours, 22 minutes	Traces	Intestine
235	4.0	2 hours	0.6	Intestine
After oral administration				
143	5.0	6 days	5.0	Feces
145	5.0	4 days	2.5	Feces
145	5.0	5 days	0.5	Feces
150}	2.0	2 days	} 0.0	Feces
150}	3.0	2 days		

* Intervals are approximate.

† Two doses of 5 mgm. each with an interval of 2 hours, 13 minutes

‡ Two doses of 5 mgm. each with an interval of 1 hour, 13 minutes.

§ Two doses of 5 mgm. each with an interval of 25 hours.

sive dose has been given, and this may account for our failure to find it in the liver after the subcutaneous injection of large doses. In one experiment three rats received a total of 9.7 mgm. of digitoxin by intravenous injection; two died in about two hours and one in about three hours; the biological test of an extract of the livers and small intestines of all three indicated the presence of about 1 mgm. of the poison. If the liver excretes digitoxin into the small intestine the excretion must take place slowly, and this may account for our failure to find even traces of the poison in the intestine in some experiments in which fairly large amounts had been given, whereas in other experiments we obtained unmistakable evidence of its presence in the intestine following its subcutaneous injection.

Table 6 shows the amounts of digitoxin administered to rats; the several modes of administration; the intervals before death or during which the urine or feces were collected; and the amounts of digitoxin recovered. A question mark is placed after two of these amounts to indicate that the tests were not wholly satisfactory. In one of these the injection of all of the available extract failed to cause perceptible effects after which the animal required an amount of ouabain nearly equal to the average fatal dose before death resulted. It is obviously impossible in such a case to determine whether the extracts contained traces of digitoxin.

Summary

The following refers only to the behavior of digitoxin in the rat.

Digitoxin is absorbed from the gastro-intestinal tract far more readily than ouabain or amorphous strophanthin, though it is practically insoluble in water.

The proportion of an oral dose of digitoxin that escapes destruction and appears in the feces varies widely in different experiments. Nearly all is destroyed in some, very little in others. This destruction is probably greater when loss of appetite prevents active intestinal peristalsis permitting a longer sojourn of the poison in the intestine.

Much the larger part of an intravenous dose of digitoxin—90 per cent or more—leaves the blood within a period of five minutes.

Part of the digitoxin injected intravenously is fixed in the liver, at least a part reaches the intestine, and a part may even appear in the feces. Much the larger part of a severely toxic intravenous dose is destroyed in the body.

Digitoxin is not excreted by the kidneys of the normal rat except possibly in traces, and it is possible that somewhat larger amounts may appear in the urine after the bile duct is tied, but this remains to be determined.

The action of a single toxic oral, subcutaneous, or intravenous dose of digitoxin is far more lasting than that of ouabain, but a very large amount of digitoxin may be survived following its subcutaneous injection in two doses administered within a short time.

The toxicity of digitoxin for the rat is about the same as that of amorphous strophanthin by intravenous injection, it is far the more toxic with oral administration; far less with subcutaneous injection.

These differences in the toxicity of the two poisons by different modes of administration depend on differences in their rates of absorption (except after intravenous injection) and elimination.

V. THE ELIMINATION OF DIGITALEIN IN THE RAT

Pure digitalein is not readily available and the commercial (crude) product is said to consist of a mixture of principles, including digitoxin, digitalin, and digitalein.¹² It is generally stated that digitoxin is rendered soluble in water by the presence of digitonin, and this commercial digitalein is readily soluble in water, affording a solution which froths on shaking.¹³

It seemed hardly worth while to conduct an extensive investigation with an impure product, such as this, but we have per-

¹² New and Nonofficial Remedies, 1918, p. 100.

¹³ Five milligrams each of digitalein and digitoxin were triturated with 25 cc. of normal saline; the resulting milky looking mixture was filtered clear and the filtrate tested on two cats. The results indicated that about 40 per cent of the digitoxin had passed into solution.

formed a few experiments and while the results of these are not especially promising they have a certain value.

Some preliminary experiments with digitalein on the rat were made by Mr. (now Professor) M. I. Smith, at that time a student in this laboratory. These experiments were conducted under the supervision of one of us (Hatcher) who assumes responsibility for the results obtained. Doses of 50 or 100 mgm. (total) were injected intramuscularly, and after periods varying from thirty to seventy-five minutes the tissues, exclusive of skin and intestine, were extracted with normal saline in the manner described for the extraction of ouabain; the extracts were tested on cats in the usual way; the results indicated the recovery of 40, 45, and 51 per cent respectively, of the amounts injected.

The urine was collected for periods of 16.5 and 20.5 hours, respectively, after the intramuscular injection of doses of 100 mgm. (total) in each of two experiments, and the urine and feces were collected and tested daily for three days after the injection of a dose of 50 mgm. In no case was the poison detected in the urine or the feces.

While digitalein is soluble in water, one should expect its separate constituents to behave independently after they have entered the blood and therefore in some experiments, made subsequent to those just mentioned, we have extracted the tissues and excreta with normal saline for the recovery of the water-soluble fraction and have then extracted the residue in the manner described for the extraction of digitoxin. Blood has been tested directly after simple dilution with normal saline. The protocol of a control experiment follows:

Ten to 20 cc. of blood were drawn from a cat into a dish containing 100 mgm. of digitalein dissolved in a few drops of normal saline, the mixture defibrinated, diluted with normal saline to make 300 cc., the proteins precipitated by heating on a water bath, the mixture filtered, and the resulting opalescent filtrate tested on cats.

The results indicated the recovery of practically all of the digitalein.

The residue left on the filter was expressed, extracted with 100 cc. of 60 per cent alcohol, the alcohol evaporated, and the residue extracted with chloroform, the liquids being allowed to stand over night. The

chloroform was evaporated and the residue dissolved in 5 cc. of 60 per cent alcohol. A test of this, on a cat showed the recovery of an amount of digitalein corresponding to that contained in the fluid remaining in the residue after expression.

Two hundred cc. of the original extract (remaining after the tests) were shaken with chloroform and allowed to stand over night. Fresh chloroform was added to the aqueous layer and allowed to stand for two days; the mixed chloroformic extracts were evaporated and the residue dissolved in 25 cc. of a mixture of normal saline and alcohol, which was tested in the usual manner.

The test indicated that the chloroformic extract contained about 8 mgm. of digitalein, or since digitalein is insoluble in chloroform, we should say that it contained the equivalent in toxicity of that amount of digitalein, in the form of a chloroform-soluble substance—probably digitoxin.

The fatal intravenous dose of digitalein for the cat is about 1.5 mgm. per kilogram, that for the rat is approximately 75 mgm. per kilogram, but individual rats show rather wide differences in tolerance; the intramuscular injection of amounts corresponding to about 600 mgm. per kilogram was survived until the animals were chloroformed after intervals of thirty minutes, one hour, and sixteen and a half hours, respectively, while one such dose was not fatal during the period of observation. The oral administration of slightly larger doses proved fatal to each of two animals after periods of about thirty hours, but one animal survived an oral dose of 725 mgm. per kilogram.¹⁴

¹⁴ The digitalein used by us was obtained in bottles, each containing a gramme or less, and all bearing the label of Merck. It was not feasible to determine the activity of each specimen, and we did not take the precaution of recording the particular bottle from which the digitalein was taken for each experiment.

When the investigation was nearly completed we found a specimen that was about twice as active as we supposed it to be. This specimen shows a fair degree of uniformity of activity when tested on the cat, except that two cats used in a series of six, died with such small doses that we must attribute their deaths to that part of the digitalein which is not a true digitalis principle. In nearly all of the experiments in which its synergism with ouabain was tested it behaved like other digitalis bodies.

A comparison of the results following its intravenous injection into rats with those observed when other specimens were used, leads us to believe that all of the digitalein used, except that employed in the earlier experiments, was of approximately the same degree of activity.

Destruction of digitalein after oral administration

Three rats, weighing 105, 131, and 137 grams, respectively received doses of 100 mgm. (total) of digitalein each, directly into the stomach. All were sick on the following day, the symptoms resembling those seen after digitoxin. The two smaller, rats died after an interval exceeding twenty-seven hours, the other one recovered.

The urine and feces of the surviving rat were collected during a period of four days, extracted together, and the extract tested on a cat by the "combined" method. The test indicated the presence of about 2 mgm. of digitalein in the total extract, but it is possible that none was present, as only about one-third of this extract was used in the test, after which the animal required 71 per cent of the average fatal dose of ouabain to cause death. A second test was not made as it was deemed of little importance whether a milligram more or less escaped destruction after the oral administration of such a large dose.

This result agrees fairly closely with those observed by Mr. Smith in the earlier experiments in which the digitalein was injected intramuscularly.

Elimination of digitalein after intravenous injection

The results of the following experiment show that digitalein, or those constituents which have a true digitalis action, leave the blood of the rat fairly promptly after intravenous injection. Two rats received intravenous doses of 8.2 mgm., and 9.4 mgm., of digitalein, respectively (corresponding to doses of 85 and 100 mgm. per kilogram). The first was exsanguinated after fifteen minutes, the second after ten minutes, the blood of each was defibrinated, diluted with normal saline, and tested on cats. All of the available blood in each case was injected into a cat, after which it required the full average fatal dose of ouabain to cause death, showing that no true digitalis principle was present in the defibrinated blood of the rats. Both of the cats used in these tests voided bloody urine just before death, this being almost certainly the result of the laking action of the digitonin

present in the digitalein. A small amount of blood was removed from the heart of one of these cats, diluted with normal saline, and allowed to stand until the corpuscles had fallen to the bottom of the vessel. The supernatant fluid was of a faint red color, showing that a slight degree of hemolysis had occurred.

The livers of these two rats were extracted, first for water-soluble principles, then for digitoxin, and the extracts tested on cats, but no evidence of the presence of any substance with a digitalis action could be obtained, the two cats took all of the extracts, and then each required the full average fatal dose of ouabain to cause death.

In another experiment a rat, weighing 174 grams, received an intravenous injection of 17.4 mgm. (total) of digitalein. Death resulted in three minutes; the animal was exsanguinated with the injection of a little normal saline, the blood was defibrinated, diluted with normal saline, and tested on two cats with fairly concordant results which indicated the presence of 8 mgm. of digitalein in the blood of the rat, or about half of the amount injected.

In a third experiment a rat weighing 98 grams received a total of 7.5 mgm. of digitalein. Death resulted in six minutes; the diluted, defibrinated blood was tested on two cats; one of these died by respiratory paralysis, which is never the cause of death when true digitalis principles are used in this way. This test, if accepted, would indicate the presence in the blood of about as much digitalein as we injected. Death was, however, probably due to the digitonin constituent. The other test animal received all of the remainder of the rat's blood—about twice as much as the first—after which it required somewhat more than the average fatal dose of ouabain to cause death, which occurred typically. We consider that the results of these tests afford conclusive evidence that any true digitalis principle (not including digitonin) present in digitalein leaves the blood of the rat fairly rapidly after its intravenous injection, but they afford no data concerning the length of time required for the digitonin constituent to leave the blood.

While we had no evidence that the liver plays any rôle in the

elimination of digitalein, we thought that we might obtain indirect evidence of its capacity for fixing and excreting the poison by tying the bile duct and examining the urine after injecting digitalein. The bile duct of each of two rats was tied during ether anesthesia, after which a total of 15.5 mgm. of digitalein was injected intravenously. The urine and feces were collected for a period of four days, extracted, and the extract tested on a cat, which received about half of the total extract, and then required the average fatal dose of ouabain to cause death, indicating that neither the urine nor the feces contained any true digitalis principles. We must suppose, therefore, that these principles are almost completely destroyed in the body after the oral, intravenous, or intramuscular administration of digitalein.

Summary

The fatal intravenous dose of the most active available commercial digitalein for the rat is roughly 75 mgm. per kilogram; that by oral or subcutaneous injection is nearly ten times as much.

Those constituents of digitalein that exert a true digitalis action leave the blood of the rat promptly, the digitonin constituent probably disappears much more slowly.

We have no evidence that the constituents of digitalein having a true digitalis action are fixed in the liver of the rat, but the question remains open.

Not more than traces of the true digitalis bodies of digitalein appear in the urine or feces after oral, intravenous, or intramuscular administration, hence they must be destroyed in the body.

We have not determined the seat of this destruction in the normal animal, but since it occurs after the bile duct has been tied, it seems probable that the liver is the chief organ concerned in the destruction of that portion which enters the circulation; the large intestine is probably concerned with the destruction of the greater part of an oral dose.

VI. THE ELIMINATION OF OUABAIN IN THE CAT AND DOG

The behavior of ouabain in the cat is so nearly like that in the dog that experiments on these two species will be considered to-

gether. The similarity of the behavior of several of the digitalis principles in the cat in man, especially with reference to their absorption from the gastro-intestinal tract, their relative toxicity after different modes of administration, the seat of their emetic action, and their persistence of action on the heart, suggests that man eliminates these principles in much the same way as the cat, hence we have made many efforts to secure convincing evidence of the behavior of the more important members of the group in the cat, but we shall here limit our discussion to the behavior of ouabain because we have been unable to devise any satisfactory method of investigating the behavior of digitoxin or other members of the group.

The want of a test animal that stands in the same relation to the cat or dog that the cat does to the rat has prevented the use of methods similar to those employed in the experiments on that animal without extensive modification, we have, therefore, resorted to the use of massive doses in many of the experiments. This affords an abundance of material for extraction, but the animals die so promptly that we are unable to determine the ultimate fate of the poison. If, on the other hand, we use small doses it is impossible to recover enough of the poison in the extracts of excreta in a form suitable for testing.

Several phases of the problem have been attacked in different ways with some overlapping in the experiments and this prevents an entirely systematic presentation of this part of the work, as that would entail duplication.

The average fatal dose of ouabain for the dog is slightly larger than that for the cat in proportion to the weight, whether the injection be made subcutaneously or intravenously, the amount required for either animal by subcutaneous injection being about one-fourth larger than that required by vein, even though the intravenous injection be extended over a period of several hours.

Since the greater part of that injected subcutaneously is absorbed within an hour or less, it is difficult to understand why so much more is required by this method of administration. One can hardly suppose that the small amount of subcutaneous tissue with which it comes in contact is capable of destroying enough of

the poison to account for this difference, and, on the other hand, it is difficult to understand how one-fifth of a subcutaneous dose can resist absorption after four-fifths have been absorbed promptly.

Absorption from the gastro-intestinal tract is so variable in both species that one can say little more about it than that the oral administration of aqueous solutions of amounts equal to five to ten times the fatal intravenous dose is usually fatal. There can be no doubt that the lower toxicity of ouabain by oral administration is due mainly to the characteristic resistance to absorption in the stomach and small intestine, seen in all species of animals (including man) in which we have studied this problem, but we have no data concerning its destruction in the large intestine of any animal except the rat.

*The elimination of ouabain from the blood of the cat and dog*¹⁵

We have extended the observation of Hatcher (27) on the rate of disappearance of ouabain from the blood of the cat and have confirmed his statement that a large part of an intravenous dose leaves the blood within a few minutes.

We have injected ouabain intravenously in the cat and dog in amounts varying from 5 to 140 times the fatal vein dose and have determined the amounts remaining in the blood at death. Death results usually in about three minutes when several times the minimal fatal vein dose is injected, and the interval before death is not materially less when an amount equal to many times the minimal fatal dose is administered.

After the injection of amounts equal to about 100 times the minimal fatal vein dose the blood, at death, usually contains about one-half to two-thirds of that injected; a somewhat smaller proportion of a still more massive dose was found in the blood in one experiment, in which the heart stopped in two minutes and twenty-three seconds; the amount found in the blood after in-

¹⁵ The cat has been used in much the larger number of our experiments of this series but we believe that the similarity of their behavior toward this poison in parallel experiments justifies our including the dog with reference to the conclusions drawn.

jections of approximately ten times the fatal vein dose averaged about one-third of that injected.

Table 7 shows the amounts of ouabain injected; the amounts found in the blood; the interval following injection before death; and the percentage of that injected which had left the blood.¹⁶

TABLE 7

Showing the rate of elimination of ouabain from the blood of the cat

AMOUNT		INTERVAL		ELIMINATED
Injected	Found in blood	Minutes	Seconds	
<i>mgm.</i>	<i>mgm.</i>			<i>per cent</i>
1.35	0.61	2	0	55
3.10	0.73	2 (about)		77
3.26	0.63	2 (about)		81
5.52	1.90	2	10	65*
7.50	1.57	2	43	79
25.00	8.10	2	40	68
25.00	9.50	1 (about)		62
25.00	7.70	2	30	69
25.00	8.90	2	23	64
25.00	9.40	2	0	62
25.00	7.70	2	0	69
37.50	15.20	2	30	60
50.00	27.40	2	23	45

* Practically all of the blood of this animal was obtained by exsanguination and subsequent perfusion.

The fixation of ouabain by the liver of the cat and dog

The rapidity with which ouabain leaves the blood indicates that it is fixed by some tissue or tissues with which the greater part of the blood comes in contact during a period of less than two minutes. The several tissues which individually meet this requirement are those of the liver, heart, lungs, brain, volun-

¹⁶ We have based our calculations on the estimate, now generally accepted by physiologists, that the blood of the cat is equal to 5 per cent of the body weight. It was formerly stated that the blood represented 7 per cent of the weight. The acceptance of this figure would indicate the recovery of a larger amount of ouabain in the blood than is shown in the table, but it is of little practical importance whether the amount of ouabain remaining in the blood after five minutes is 30 per cent or 40 per cent of that injected.

tary musculature, gastro-intestinal tract, and perhaps the kidneys. We have investigated these successively in the effort to determine the part which each plays in removing the poison from the blood. For convenience the blood vessels are considered as a part of the tissues which they supply and from which they cannot be separated, but we have endeavored to learn whether they fixed ouabain during perfusion with it.

The observation that the liver of the rat plays such an important rôle in the elimination of ouabain inclined us to the view that this organ is also concerned in its elimination in other species of animals, but, on the other hand, it is plain that the liver of the cat or dog is incapable of protecting it against such large doses as the rat withstands, hence we did not expect to find the poison fixed in the liver in large amounts.

Massive doses of ouabain were injected intravenously into cats, the animals were exsanguinated at death, or within a few minutes thereafter, the liver, previously rendered nearly bloodless in some cases, was extracted and the extract tested on cats in the usual manner.

None of the poison was recovered from the liver in some experiments; in others small amounts were recovered, but in no instance were these sufficient to account for more than a fraction of that which had left the blood.

The protocol in brief of one of these experiments follows.

A cat, weighing 3.94 kgm, received 25 mgm. ouabain into the femoral vein; the heart stopped after two minutes; the portal vein, and the vena cava above the liver, were tied; the liver was removed and drained of blood; this, and that drained from the remainder of the body, were defibrinated separately, each was diluted with normal saline and tested in the usual manner.

After the blood had been drained from the liver it was perfused with enough 2 per cent solution of sodium chlorid to furnish 200 cc. of perfused fluid; it was then perfused with normal saline until 280 cc. of the second perfused fluid were obtained. This second perfused fluid was almost colorless.

The liver, weighing 75 grams, was extracted in the usual manner with 300 cc. of normal saline, and the several fluids were separately tested.

The results of the tests indicated the presence of the following amounts of ouabain:

Total blood of the body	8.40 mgm.
Blood drained from liver, 7.5 cc.	0.35 mgm.
First perfused fluid from liver, 200 cc.	0.92 mgm.
Second perfused fluid from liver, 280 cc.	0.30 mgm.
Extract of liver, 375 cc. (including liver tissue)	1.25 mgm.

It is sometimes stated that the liver contains about one-fourth of the total blood of the animal, however, we were able to obtain only 7.5 cc. by simple drainage in this experiment, but practically all of the remainder was removed by perfusion with the first portion of 200 cc. of fluid, which yielded approximately 1 mgm. of ouabain; the second perfused fluid contained much less than one-third as much blood as the first, since it was nearly colorless, but it contained about as much ouabain as the 7.5 cc. of pure blood, and we must suppose that a portion of this amount of the poison was removed from the liver cells or from the blood vessels of that organ. The liver tissue was then practically bloodless, but the extract contained 1.25 mgm. of ouabain.

It may be seen from the summary of results of this experiment that the total amount of ouabain recovered from the liver, including that from its blood, the first and second perfusions, and the extract, was 2.82 mgm., an amount almost precisely equal to one-third of that indicated in the total blood of the animal. This would seem to confirm the view that the liver did contain one-fourth of the total blood and that nearly all of the ouabain recovered from that organ was present in the contained blood, but the fact that the second perfusion fluid, though almost entirely free of blood, contained about as much of the poison as the 7.5 cc. of pure blood drained from the liver, and the further fact that the practically blood-free liver contained as much of the poison as this pure blood and the first perfused fluid together disprove any such contention. A liver weighing 75 grams could not retain half of its blood after having been drained as far as possible and then perfused until the fluid was nearly colorless, nor could it yield a nearly colorless extract with so much blood present.

We found the concentration of the poison to be the same in the blood of the liver and that of the remainder of the body in two of the experiments in which comparative tests were made. It might be argued from this that the liver does not fix the poison. However, the greater part of the blood of the cat passes through the liver several times within a period of two minutes and we have no means of detecting minute differences in concentration of the poison, such as must exist in the blood of different regions; this similarity in concentration of the poison in the blood of different parts of the body suggests its fixation by more than one organ or tissue (cf. results of experiments on rats). In one experiment the concentration in the blood of the liver was much less than that in the remaining blood.

We thought at times that we failed to recover from the liver all of the ouabain present; we therefore resorted to feeding experiments in the belief that any stable combination of the ouabain with the liver cells would be broken down during digestion and the ouabain then absorbed. We did not expect to be able to estimate accurately the amount of ouabain present in the liver in this way (because of the variability in the rate of its absorption from the gastro-intestinal tract), but we anticipated that we could secure qualitative evidence of its fixation after the intravenous injection of massive doses, which were equal to many times the fatal oral dose when administered in pure aqueous solution.

Arnaud (3) reported that ouabain is non-toxic when administered orally, and this statement has been repeated frequently by other investigators, but it is well known that the tolerance of the cat and dog toward pure solutions of ouabain, administered orally, is strictly limited, although, as previously stated, the rate of absorption is quite variable. The oral administration of an amount equal to ten times the fatal vein dose almost invariably gives rise to toxic symptoms, and in these feeding experiments we had administered amounts equal to one hundred times the fatal vein dose.

Massive doses of ouabain were injected intravenously into cats; at death the livers were excised and fed to other cats, some

of which vomited, while others showed no effects. In control experiments large amounts of ouabain were added to meat and liver, which were fed to cats and dogs, but in no instance were we able to kill either of these animals in this way, even when the amounts of ouabain employed were equal to forty times the fatal intravenous dose. Some of the animals showed no perceptible effects, others vomited, but the emesis may have been due to the raw meat or liver.

The livers of cats and dogs were perfused in the effort to determine whether that organ is capable of fixing or destroying the poison. The results of some of these experiments were negative, but in others the evidence of fixation was convincing; however, in no case was the amount fixed comparable to that which leaves the blood within a few minutes after the intravenous injection of a massive dose. We must suppose, therefore, that in the cat and dog the liver has the power of fixing a small amount of ouabain, but that this capacity is far less than that of the liver of the rat.

An abstract of the protocol of one of these experiments will be given and the essentials of the series will be discussed, but it is not convenient to tabulate the results of all of them because of the variety of conditions under which the perfusions were carried out.

A cat, weighing 3.22 kgm., was exsanguinated during the injection of 150 cc. of Locke's solution, 230 cc. of blood and fluid being obtained. This was defibrinated, strained, and to it were added 10 mgm. of ouabain and enough normal saline to make 250 cc.

This fluid was perfused about twenty-five times through the portal circulation of the excised liver, with frequent oxygenation, during two hours at a temperature of about 37°C.

The perfused fluid then measured 230 cc., after which the liver was perfused with enough Locke's solution to obtain an additional 265 cc.

The liver, weighing 55 grams, was extracted with 220 cc. of normal saline. The total extract was calculated as 275 cc., the liver being considered as though it were fluid.

Each of the several fluids was then tested on two cats; the following being a summary of the results:

First perfused fluid, 230 cc.	4.50 mgm. ouabain
Second perfused fluid, 265 cc.	0.80 mgm. ouabain
Extract of liver, 275 cc.,	<u>2.75</u> mgm. ouabain
Total	8.05 mgm. ouabain

It will be seen from the foregoing protocol that the fluid remaining at the end, was less by only 20 cc. than at the beginning, of perfusion, but it contained only about half of the ouabain which had been added, while a larger volume of the second perfused fluid contained about one-sixth as much as the first. It is impossible to say whether the small amount present in this fluid was removed from a loose combination in the vessel walls, or by washing out the residuum of the first perfused fluid which had become mixed, by diffusion, with the fluids of the liver. There is no reason to doubt, however, that the liver had actually fixed a considerable proportion of the ouabain, and it had probably destroyed a smaller amount.

The liver of a dog was perfused for three hours with 1000 cc. of a mixture of defibrinated blood and Locke's solution to which 25 mgm. of ouabain had been added, after which 925 cc. of perfused fluid were obtained, the liver having become swollen. It was then perfused once with 500 cc. of Locke's solution after which the liver was extracted in the usual way. Tests of the several fluids indicated the presence of the following amounts of ouabain:

First perfused fluid, 925 cc.	12.2 mgm.
Second perfused fluid, 500 cc.	1.5 mgm.
Extract of liver	<u>4.2</u> mgm.
Total	17.9 mgm.
Difference	7.1 mgm.

In another experiment, conducted in an essentially similar way, the perfused fluid contained 80 per cent of the ouabain which had been added, and the test of the extract of the liver failed to show that any of the poison had been fixed by it, but since we neglected to use the "combined ouabain" method in this experiment it is possible that the liver may have contained a small amount.

These experiments show clearly that some of the ouabain was fixed in the liver. Had it been present in loose combination in the walls of the vessels alone it would have been removed during perfusion with the Locke's solution, as indeed, a part of it appears to have been. But it is possible that that which was extracted from the liver required the use of heat in order to render it soluble. It seems much more probable to us, however, in view of the functions of the liver, that the ouabain extracted had been taken up by the cells of that organ and was in process of elimination, either by means of excretion in the bile or by destruction, and it should be stated that in only one or two experiments were we able to recover nearly all of the ouabain added to the perfused fluid.

Several of the perfusion experiments do not call for detailed discussion owing to the lack of sufficient data, such for example, as in the last experiment detailed, or because of failure to secure a satisfactory perfusion.

It may be merely a coincidence that the highest percentages of ouabain recovered in these perfusion experiments, with one exception, correspond closely with the highest percentages recovered from the rat after intravenous injection.

Having found that the removal of the liver, or tying the portal vein, lessens the resistance of the rat to ouabain, we endeavored to learn whether a similar procedure would have a corresponding effect in the cat, although it had been previously shown that no more ouabain was required to kill the dog when it was injected into the portal, than when it was injected into the femoral vein.

In a series of three experiments, in which the celiac axis, the superior mesenteric artery, and portal vein had been tied previous to the injection of ouabain into the femoral vein, the animals died after the administration of amounts equal to 50, 60, and 70 per cent, respectively, of the average fatal intravenous dose for the normal animal. This would seem to indicate that the liver does exert a considerable degree of protection in the cat, but control experiments showed that after the exclusion of other large vascular areas similar reductions occurred in the amounts of ouabain required to kill the cats. In another experiment the superior mesenteric artery, the aorta (just below the axis), and the

vena cava at the level of the axis, were tied. A dose of 25 mgm. of ouabain was injected into the external jugular vein. The heart stopped in two minutes and forty-one seconds, the animal was exsanguinated, while normal salt solution was being injected into the vena cava, the carotid, and the arch of the aorta, 1040 cc. of fluid being obtained. This was found to contain 9.3 mgm. of ouabain, nearly 16 mgm. having left the blood stream in the restricted area when neither the liver nor the gastro-intestinal tract participated in its removal. This shows that other tissues removed ouabain from the blood, and it suggests the muscle or brain as the seat of its fixation.

While the results just mentioned show that ouabain disappears rapidly from the blood without the participation of the gastro-intestinal tract, except in so far as a small amount of blood reaches it through the collateral circulation, we have removed various abdominal organs and tied the vessels supplying other tissues before injecting the poison, in the further effort to determine how it is removed from the circulation. An abstract of the protocol of this experiment shows that despite the extraordinary degree to which the circulation was restricted—virtually to the heart, lungs, head, and thorax—the poison disappeared from the blood almost as rapidly as in the intact animal.

A cat, weighing 4.46 kgm., was anesthetized, and the gastrointestinal tract from the esophagus to the rectum, the kidneys, spleen, and pancreas were removed. The subclavian arteries and veins, the celiac axis, the superior mesenteric artery, the portal vein, the vena cava, above the bifurcation, and the renal arteries and veins were tied.

The animal received 25 mgm. of ouabain intravenously and the heart stopped after two minutes and thirty-three seconds.

The body was exsanguinated so far as possible by injecting normal saline into the cava and other veins, and into the carotid.

The bloody fluid thus obtained measured 1340 cc., and it was estimated that it contained 100 cc. of blood, or about one-third of the total in the animal.

The tests indicated that about half of the ouabain injected had disappeared from the blood of this restricted area.

Fixation in the brain

It is a curious fact that while numerous investigators have attempted to recover various digitalis principles from the hearts of different animals and to estimate them quantitatively, few have attempted to recover these principles from the central nervous system, although their action on the respiratory and vomiting centers in the medulla is as characteristic as that on the heart, and there is abundant clinical testimony that they also act on the cerebrum. We therefore looked to the central nervous system as the probable seat of fixation of large amounts of ouabain in the cat and dog, especially in view of the fact that the poison disappeared from the blood in the experiment just detailed.

The intravenous injection of an amount of ouabain equal to 65 per cent of the average fatal dose caused cardiac standstill in a dog in which the cerebral circulation was interfered with by previously tying the carotid and vertebral arteries; and a somewhat larger relative amount was required to stop the heart of a decapitated dog (hemorrhage having been arrested) during artificial respiration.

The diminution in the amounts required in these experiments is probably explained by the diminished vascular area, but we perfused the brain of a cat with defibrinated blood to which ouabain had been added in order to determine whether fixation occurs. The results were negative and we then attempted to learn whether the intact brain of the animal fixes the poison after its intravenous injection.

In this experiment the abdominal aorta and vena cava were tied below the level of the renals, and 25 mgm. of ouabain were injected into the vena cava toward the heart; the latter ceased to beat in two minutes. The animal was then exsanguinated during the injection of normal saline, 1200 cc. of bloody fluid being obtained. The tissues of the liver, brain and muscles were then pale and practically bloodless. The brain, including the medulla and cerebellum, was extracted with enough normal saline to make 500 cc., and this and the defibrinated bloody fluid were tested. The tests indicated that the blood contained 9 mgm. of ouabain,

and that about 16 mgm. had left the circulation. The extract of the brain (which did not show a trace of color) contained no ouabain whatever. The test animal received 25 cc. of the extract per kilogram, after which it required a little more than the average fatal dose of ouabain to cause death. A control experiment showed that ouabain could be extracted after its injection into the excised brain and estimated quantitatively.

This failure to recover ouabain from the blood-free brain is not incompatible with the fact that it exerts a typical action on that organ, since mere traces, far too small for us to detect, may be sufficient to exert its peculiar effects, especially those on the respiratory and vomiting centers.

Fixation in muscle

We next investigated the rôle of the voluntary muscles in the removal of ouabain from the blood. A cat received an intravenous injection of 25 mgm. of ouabain, and died in two minutes. The test of the defibrinated blood indicated that about two-thirds of the poison injected had left the circulation. One hundred grams of muscle, taken from the thigh, were extracted and the extract tested on two cats, each of which received an amount of the extract corresponding to 10 grams of muscle for each kilogram of weight, after which it required almost the exact average fatal dose of ouabain to cause death. This result is the more surprising in that the muscle must have contained at least a small amount of blood, hence we must suppose that the extract contained at least traces of the poison. It will be remembered that the muscle of the rat was also found to be free of ouabain after the injection of a large dose, but that may be explained partly by the greater capacity of the liver of the rat to fix the poison.

A practically similar result was obtained in another experiment in which 37.5 mgm. of ouabain were injected intravenously into a cat weighing 3.75 kgm. The cat was bled, but only 75 cc. of the blood, or less than half of the calculated total amount in the body, were obtained. The test indicated that about 22 mgm. of ouabain had left the circulation. An extract of 423 grams of the muscles of the thighs was made and tested, the result indi-

cating the presence of about 2 mgm. of ouabain in the total extract; and since so little blood had been removed, when exsanguination was attempted, we must suppose that all, or nearly all, of the 2 mgm. obtained was present in the blood in the muscles.

The hind limbs of a cat were perfused during seventy minutes with a mixture of defibrinated blood and Locke's solution to which 10 mgm. of ouabain had been added. The test of the perfused fluid indicated that very little, if any, of the ouabain had been removed from the fluid during perfusion. We must conclude, therefore, that the voluntary muscles do not fix more than traces of ouabain, during perfusion, at least.

Fixation in the kidneys

It seemed probable to us that the kidneys excrete at least a part of the ouabain administered, but we have been unable to detect such small amounts as may be eliminated in the urine of the dog because the toxicity of the urine is greater than that of any ouabain which it contains and we have no satisfactory means of separating it from the urine. We attempted to determine whether the poison is excreted in amounts which can be estimated in the urine of the dog during diuresis, induced by hypertonic sodium sulphate solution. The urine then secreted being of very low toxicity in proportion to its volume.

Two experiments were carried out on small dogs, each of which received an intravenous injection of 0.5 mgm. of ouabain. The urines were collected during two hours, and two hours and twenty minutes, respectively, and tested on cats by the "combined ouabain" method. The tests indicated the excretion of about 0.04 mgm. of ouabain in the first dog, and of about half as much in the second, in which diuresis was much less than in the first.

The excised kidney of a cat was perfused once with one part of ouabain in two hundred and fifty thousand parts of Locke's solution, after which the fluid was tested on a cat by the "combined ouabain" method, the test indicating that about 7 per cent of the ouabain had been removed from the perfused fluid. Such minute amounts cannot be estimated accurately, however.

Fixation in the heart and lungs

One seldom thinks of the lungs as fixing poisons of this type and of removing them from the blood, but we wished to secure direct evidence concerning their capacity for fixing ouabain. Previous investigators had shown that the heart does not fix important amounts of ouabain; we therefore employed a method in which the circulation is limited to the heart and lungs in order to determine the rôle of the lungs in its fixation.

The protocol, in brief, of an experiment follows. It indicates that not more than traces of the poison is fixed in the heart or lungs. In another experiment of this type, in which 5 mgm. of ouabain were injected, the test indicated the recovery of 80 per cent of the poison, and it is possible that even that estimate is too low.

A cat, weighing about 4 kgm., was etherized and given artificial respiration. The innominate artery and superior vena cava were connected by a tube and cannulas, after which the aorta was tied just beyond the origin of the innominate, and the inferior vena cava, close to the heart.

Ten mgm. of ouabain were injected into the chamber of the left ventricle; the heart stopped after one minute and forty-five seconds. The heart and lungs were then exsanguinated during the injection of enough normal saline to yield 300 cc. of blood and fluid. The vessels and lungs were cut, in order to exsanguinate the tissues more completely, while enough normal saline was being injected to yield 700 cc. of additional fluid.

The tests of these fluids gave the following results:

First fluid, 300 cc.,	8.00 mgm. ouabain
Second fluid, 700 cc.	1.88 mgm. ouabain
Total	<hr/> 9.88 mgm. ouabain

Having failed to detect more than traces of ouabain in the urine and various bloodless tissues, except the liver, after the intravenous injection of massive doses, we considered an attempt to extract the entire body of the cat (exclusive of the skin and intestinal contents) after the injection of a large dose of the poison,

but we decided that the method which we use would not suffice for its estimation in such dilute solutions as the extracts would yield. We therefore abandoned the plan.

Summary

Ouabain disappears rapidly from the circulation of the cat and dog, less than 50 per cent of a massive intravenous dose being present in the blood at death, which occurs within two to three minutes.

Small amounts of ouabain can be detected in the nearly bloodless liver after the administration of such doses, and after perfusion with diluted, defibrinated blood to which ouabain has been added.

The participation of the liver is not essential for the rapid removal of ouabain from the blood, for it disappears almost as rapidly when the circulation is restricted to certain small areas, but not when it is limited to the heart and lungs.

It is probable that small amounts of ouabain are excreted by the kidneys, but it cannot be detected in the urine of the cat or dog after the administration of sub-lethal doses, except possibly after copious diuresis.

Ouabain cannot be detected in the bloodless voluntary muscle or brain after the intravenous injection of massive doses, or in those structures after perfusion with it.

We can offer no other explanation of the rapid disappearance of large amounts from the blood than that the normal liver decomposes the poison more rapidly than the perfused liver, or, that the ouabain is widely distributed in various tissues of the body.

If that part of the ouabain which leaves the blood before death were distributed nearly uniformly throughout the body, the amount present in any one tissue would be insufficient to permit of its extraction and estimation (owing to its dilution in the extract) with a sufficient degree of precision to permit of our determining whether it was present in the blood of the tissues or in the tissues themselves. Our inability to detect such amounts

in extracts of the tissues will be understood when it is remembered that the fatal intravenous dose of ouabain for the cat is only equal to one-ten-millionth of the body weight.

REFERENCES

- (1) HATCHER: *Journ. Am. Pharm. Assoc.*, v, 157, 1916.
- (2) CATILLON: *Bul. et Mem. de la Soc. Thérap.*, Paris, p. 217, 1887.
- (3) ARNAUD: *Comptes rendus de l'Acad. des Sci.*, cvi, 1011, 1888; cvii, 1162.
- (4) THOMS: *Ber. de deut. pharm. Gesell.*, xiv, 104, 1904.
- (5) HEDBOM: *Arch. exp. Path. u. Pharm.*, xlv, 317, 1901.
- (6) KOELLICKER: *Verhand. der phys. med. Gesellschaft, Wurzburg*, viii, 284, 1858.
- (7) POLAILLON AND CARVILLE: *Arch. Phys. norm. et path.*, iv, 523, 680, 1871-1872.
- (8) HEUSER: *Arch. int. de Pharm. et de Thérap.*, x, 483, 1902.
- (9) CLOETTA AND FISCHER: *Arch. exp. Path. u. Pharm.*, liv, 294, 1906.
- (10) KILIANI: *Muench. med. Woch.*, i, 886, 1907.
- (11) YERNAUX: *Arch. int. de Pharm. et de Thérap.*, xviii, 117, 1908.
- (12) WERSCHININ: *Arch. exp. Path. u. Pharm.*, lx, 328, 1909.
- (13) WERSCHININ: *Arch. exp. Path. u. Pharm.*, lxiii, 386, 1910.
- (14) SCHLIOMENSUN: *Arch. exp. Path. u. Pharm.*, lxiii, 294, 1910.
- (15) OPPENHEIMER: *Biochem. Zeit.*, lv, 134, 1913.
- (16) HATCHER: *Am. J. Phys.*, xxiii, 303, 1909.
- (17) HATCHER AND BAILEY: *J. Am. Med. Assoc.*, lii, 5, 1909.
- (18) DALE AND LAIDLAW: *Heart*, i, no. 2, 1909.
- (19) FOCKE: *Therap. Monats.*, xxv, 533, 1911.
- (20) STRAUB: *Biochem. Zeitsch.*, No. 28, 392, 1911.
- (21) ISSEKUTZ: *Arch. exp. Path. u. Pharm.*, lxxviii, 155, 1915.
- (22) GRUENWALD: *Arch. exp. Path. u. Pharm.*, lxviii, 231, 1912.
- (23) WALLER, A. D.: *Jour. Phys.*, xxxviii, 1909; *Proc. Phys. Soc.*, p. x.
- (24) STRAUB: *Arch. exp. Path. u. Pharm.*, lxxi, 139, 1913.
- (25) WEISZAECKER: *Arch. exp. Path. u. Pharm.*, lxxii, 347, 1913.
- (26) LHOTÁK: *Biochem. Zeitsch.*, xlviii, 144, 1913.
- (27) HATCHER: *J. Am. Med. Assoc.*, lxi, 386, 1913.
- (28) CLARK: *J. Pharm. and Exp. Therap.*, iv, 399, 1913.
- (29) GROEBER: *Arch. exp. Path. u. Pharm.*, lxxii, 282, 1913.
- (30) DEUCHER: *Deutsch. Arch. f. klin. Med.*, lvii, 1, 1896; lviii, 47.
- (31) KRAUSE: *Arch. f. Schiffs-und Tropenhygiene*, x, 105, 1906.
- (32) LEOWY: *Wien. klin. Woch.*, xix, 1157, 1906.
- (33) EGGLESTON and HATCHER: *J. Am. Med. Assoc.*, lv, 1902. 1915.
- (34) HATCHER: *Am. J. Phys.*, xxiii, 303, 1909.
- (35) HALE, WORTH: *J. Am. Med. Assoc.*, lvii, 1515, 1911.
- (36) LHOTÁK: *Arch. int. de Pharm. et de Thérap.*, xxii, 61, 1912.
- (37) HATCHER: *Arch. int. Med.*, x, 268, 1912.
- (38) HOLSTE: *Arch. exp. Path. u. Pharm.*, lxviii, 323, 1912.
- (39) JOHANNESSEN: *Arch. exp. Path. u. Pharm.*, lxxviii, 83, 1914.
- (40) HATCHER and EGGLESTON: *J. Pharm. and Exp. Therap.*, x, 281, 1917.

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